Dicamba and Metabolites DCSA and DCGA

Mammalian Toxicology
Summaries and Assessment
(Tier II)

Trilateral Joint Review
United States Environmental Protection Agency
Health Canada Pest Management Regulatory Agency
Japan Food Safety and Consumer Affairs Bureau

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IIA 5.8.3 Oral

Report:	IIA 5.8.3/02 Smedley, J. (2007) An Acute Oral Toxicity Study in Rats with MON 52708. Charles River Laboratories, Spencerville, Ohio, US. Study Number: EUF00137. Issue date 11 January 2007. Unpublished. (Monsanto Study No.: CRO-2006-050). MRID #47899504.

Guidelines: Acute Oral Toxicity (rat) OECD 425 (2001): OPPTS 870.1100 (2002)

Sponsor: Monsanto Company

Executive Summary: In an acute oral toxicity study (Up and Down Procedure), young adult female Sprague Dawley rats were given a single oral dose of MON 52708, a metabolite of Dicamba, at 550 mg/kg bw, 2000 mg/kg bw or 5000 mg/kg bw. The test substance was administered as a 25% w/w mixture in distilled water. Animals were observed for mortality, signs of gross toxicity, and behavioural changes at least once daily for 14 days or until death. All animals were necropsied.

The single animal dosed at 550 mg/kg bw survived, gained weight, and exhibited no clinical signs of toxicity during the study period. No gross lesions were observed at necropsy

Two of the five animals dosed at 2000 mg/kg bw died within one day post-dosing. Clinical observations noted prior to death included wobbly gait, rapid breathing, urine/fecal stain, apparent hypothermia, ocular discharge, dark material around the nose, intermittent tremors, soft stools and salivation. In surviving animals, clinical observations were limited to transient incidences of urine stain, dark material around the nose and excessive food pile under the cage in one animal. Survivors gained weight throughout the study period. At necropsy, abnormal content of the digestive tract was observed in decedents. One incidence of a cyst on the uterus was noted in one animal that survived to study termination; this finding was not considered to be related to the test substance due to the isolated nature of the finding.

Three of the four animals dosed at 5000 mg/kg bw died post-dosing on day 0. Clinical observations noted prior to death included tremors, prostration, laboured breathing, fecal stain, apparent hypothermia, ocular/nasal discharge, wobbly gait, decreased activity, soft stools and salivation. In the single surviving animal, clinical observations were limited to one incidence of dark material around the nose. The survivor gained weight throughout the study period. At necropsy, abnormal content of the digestive tract, stomach discolouration, slight autolysis of most organs of the abdominal cavity, and foci on the thymus were observed in decedents. No gross lesions were observed in the lone survivor.

Oral LD_{50} Females = 2641 mg/kg bw (based on maximum likelihood)

Based on an estimated LD_{50} of 2641 mg/kg bw in female rats, MON 52708 meets the criteria for USEPA Toxicity Category III.

This acute oral study is classified as Acceptable. This study satisfies the guideline requirement for an acute oral study (OPPTS 870.1100; OECD 425) in the rat.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. Materials:

Test Material:	MON 52708
Description:	White powder
Lot No./:	GLP-0603-16958-T
Purity:	97.9%
CAS#:	Not reported
Vehicle:	Distilled water
Stability of test compound:	Expiration date: 20 March 2007

Test Animals:	
Species	Rat
Strain	Sprague Dawley
Age at dosing	8-10 weeks
Weight at dosing	180-213 g females
Source	Harlan Sprague Dawley, Inc., Indianapolis, Indiana, US
Housing	Singly housed in suspended stainless steel cages.
Acclimatization period	at least 5 days
Diet	PMI Certified Rodent Chow #5002
Water	Municipal tap water treated by reverse osmosis, *ad libitum*
Environmental conditions	Temperature: 18-23°C Humidity: 36-71 % Air changes: 10-15 per hour Photoperiod: 12 hour light/12 hour dark

B. Study Design and Methods:

1. **In-life dates:** Start: 4 May 2006 End: 16 June 2006

2. **Animal assignment and treatment:** Prior to each dosing, experimentally naïve rats were fasted and weighed. Ten healthy naïve rats were selected for test. Animals were assigned to the test groups as noted in Tables IIA 5.8.1/02-1, below. The test substance was administered as a 25% w/w mixture in distilled water via gavage. Initially, the test substance was administered to a single female at a dose of 2000 mg/kg bw. Following the Up and Down procedure, nine additional animals were dosed at either 550 mg/kg bw, 2000 mg/kg bw or 5000 mg/kg bw. The test substance was administered in sequence. Individual body weighs were recorded prior fasting (day -1), prior to dosing on (day 0) and again on Days 7 and 14or after death. Animals were observed for clinical abnormalities a minimum of two times on day 0 post-dosing, with the first observation

within 30 minutes post-dosing, and daily thereafter for the 14 day study period. All animals were necropsied.

Table IIA 5.8.3/02-1. Main Test: Doses, mortality/animals treated

Dosing Sequence	Animal No.	Sex	Dose (mg/kg bw)	24 Hour Result	48 Hour Result
1	A5923	F	2000	X	X
2	A5926	F	550	O	O
3	A5995	F	2000	O	O
4	A5986	F	5000	X	X
5	A6075	F	2000	O	O
6	A6045	F	5000	O	O
7	A6078	F	5000	X	X
8	A6094	F	2000	O	O
9	A6087	F	5000	X	X
10	A6166	F	2000	X	X

(O = survived, X = dead)

3. Statistics: After each animal was dosed, the short-term and long-term outcomes (mortality) were input into the OECD 425 Acute Oral Toxicity Statistical Program (OECD 425 AOT Program). When the stopping criteria were engaged, the program calculated the LD50 and 95% confidence intervals.
Body weight means and standard deviations were calculated.

II. RESULTS AND DISCUSSION

A. Mortality: The single animal dosed at 550 mg/kg bw survived. Two of the five animals dosed at 2000 mg/kg bw died within one day post-dosing. Three of the four animals dosed at 5000 mg/kg bw died post-dosing on day 0.

B. Clinical observations: The single animal dosed at 550 mg/kg bw exhibited no clinical signs of toxicity during the study period.
Clinical observations noted prior to death in animals dosed at 2000 mg/kg bw included wobbly gait, rapid breathing, urine/fecal stain, apparent hypothermia, ocular discharge, dark material around the nose, intermittent tremors, soft stools and salivation. In surviving animals, clinical observations were limited to transient incidences of urine stain, dark material around the nose and excessive food pile under the cage in one animal.
Clinical observations noted prior to death in animals dosed at 5000 mg/kg bw included tremors, prostration, laboured breathing, fecal stain, apparent hypothermia, ocular/nasal discharge, wobbly gait, decreased activity, soft stools and salivation. In the single surviving animal, clinical observations were limited to one incidence of dark material around the nose.

C. Body weight: Survivors gained weight throughout the study period.

D. Necropsy: No gross lesions were observed at necropsy in the single animal dosed at 550 mg/kg bw.

At necropsy, abnormal content of the digestive tract was observed in decedents dosed at 2000 mg/kg bw. One incidence of a cyst on the uterus was noted in one animal that

survived to study termination; this finding was not considered to be related to the test substance due to the isolated nature of the finding.

At necropsy, abnormal content of the digestive tract, stomach discolouration, slight autolysis of most organs of the abdominal cavity, and foci on the thymus were observed in decedents dosed at 5000 mg/kg bw. No gross lesions were observed in the lone survivor.

E. Investigator's Conclusions (extracted from page 16 in the study report): "*Under the conditions of this test, the acute oral LD50 of MON 52708 was estimated to be 2641 mg/kg (based on maximum likelihood) in the rat.*"

F. Reviewer's Conclusions: The reviewer is in agreement with the investigators. Based on an estimated LD_{50} of 2641 mg/kg bw, MON 52708 meets the criteria for USEPA Toxicity Category III.

G. Deficiencies: No deficiencies were identified.

IIA 5.8.3 Oral

Report:	IIA 5.8.3/01 Oley, S. (2009) Acute Oral Toxicity Up and Down Procedure in Rats. Eurofins|Product Safety Laboratories, Dayton, New Jersey, US. EPSL Study Number: 27301. Issue date 16 July 2009. Unpublished. (Monsanto No. EPS-09-076). MRID #47899505.

Guidelines: Acute Oral Toxicity (rat) OECD 425 (2001): OPPTS 870.1100 (2002)

Sponsor: Monsanto Company

Executive Summary: In an acute oral toxicity study (Up and Down Procedure), young adult female Sprague-Dawley rats were given a single oral dose of MON 52724, a metabolite of Dicamba, at a dose of 820 or 2600 mg/kg bw. The test substance was administered as a 35% w/v mixture in distilled water. Animals were observed for mortality, signs of gross toxicity, and behavioural changes at least once daily for 14 days or until death. All animals were necropsied.

Oral LD_{50} Females = 1460 mg/kg bw (95% C.I. = 820 - 2600 mg/kg bw)

All animals dosed at 820 mg/kg bw (3 animals) survived and gained body weight throughout the study period. Post-dosing, two animals exhibited piloerection and/or reduced fecal volume; however, these animals recovered by Day 2 and along with the other animal appeared active and healthy for the remainder of the observation period. No gross lesions were observed at necropsy.

All animals dosed at 2600 mg/kg bw (3 animals) died within one day post-dosing. Prior to death, these animals were hypoactive and exhibited piloerection and/or hunched posture. At necropsy, red intestines were observed in all decedents.
Based on an estimated LD_{50} of 1460 mg/kg bw in female rats, MON 52724 meets the criteria for USEPA Toxicity Category III.

This acute oral study is classified as Acceptable. This study satisfies the guideline requirement for an acute oral study (OPPTS 870.1100; OECD 425) in the rat.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. Materials:

Test Material:	MON 52724
Description:	Off-white powder
Lot/EPSL Reference number:	GLP-0903-19699-T/090403-2R
Purity:	96.3%
CAS#:	Not reported
Vehicle:	Distilled water
Stability of test compound:	Expected to be stable for the duration of testing. Expiration date: 12 March 2010

Test Animals:	
Species	Rat
Strain	Sprague-Dawley
Age at dosing	9-10 weeks
Weight at dosing	172-184 g females
Source	Ace Animals, Inc., Boyertown, Pennsylvania, US
Housing	Singly housed in suspended stainless steel caging with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
Acclimatization period	6-14 days
Diet	Purina Rodent Chow (#5012)
Water	Filtered tap water *ad libitum*
Environmental conditions	Temperature: 19-21°C Humidity: 30-68 % Air changes: not reported Photoperiod: 12 hour light/12 hour dark

B. Study Design and Methods:

1. In-life dates: Start: 13 April 2009 End: 4 May 2009

2. Animal assignment and treatment: Prior to each dosing, experimentally naïve rats were fasted overnight and examined for health and weighed (initial). Six healthy, naïve rats were selected for test. Animals were assigned to the test group as noted in Table IIA 5.8.1/01-1, below. The test substance was administered as a 35% w/w mixture in distilled water via gavage at a dose of 820 or 2600 mg/kg bw. Initially, the test substance was administered to a single female at a dose of 820 mg/kg bw. Based on the survival of this animal, five additional females received a dose of either 820 or 2600 mg/kg bw. The decision to proceed with the next animal was based on the survival of the previous animal post-dosing.

Individual body weighs were recorded prior to test substance application (initial) and again on Days 7 and 14 (termination). Animals were observed for mortality, signs of gross toxicity, and behavioural changes during the first several hours post-dosing and at least once daily thereafter for the 14 day study period. All animals were necropsied at study termination.

Table IIA 5.8.3/01-1. Doses, mortality/animals treated (O = survived, X = dead)

Dosing Sequence	Animal No.	Sex	Dose (mg/kg bw)	24 Hour Result	48 Hour Result
1	3101	F	820	O	O
2	3102	F	2600	X	X
3	3103	F	820	O	O
4	3104	F	2600	X	X
5	3105	F	820	O	O
6	3106	F	2600	X	X

3. Statistics: The *Acute Oral Toxicity (Guideline 425) Statistical Program* (Weststat, version 1.0, May 2001) was used for all data analyses including: dose progression selections, stopping criteria determinations and/or LD50 and confidence limit calculations.

II. RESULTS AND DISCUSSION

A. Mortality: All 3/3 animals survived at a dose of 820 mg/kg bw. All 3/3 animals died within one day post-dosing at 2600 mg/kg bw.

B. Clinical observations: Post-dosing, two of three animals dosed at 820 mg/kg bw exhibited piloerection and/or reduced fecal volume; however, these animals recovered by Day 2 and along with the other animal appeared active and healthy for the remainder of the observation period.

Prior to death, animals dosed at 2600 mg/kg bw were hypoactive and exhibited piloerection and/or hunched posture.

C. Body weight: All surviving animals gained body weight throughout the study period.

D. Necropsy: No gross lesions were observed at necropsy in survivors. At necropsy, red intestines were observed in all decedents.

E. Investigator's Conclusions (extracted from page 13 in the study report): "*Under the conditions of this study, the acute oral LD_{50} of MON 52724 is estimated to be 1460 mg/kg of body weight in female rats (based on an assumed sigma of 0.5) with an approximate 95% PL confidence interval of 820 mg/kg (lower) to 2600 mg/kg (upper).*"

F. Reviewer's Conclusions: The reviewer is in agreement with the investigators. Based on an estimated LD_{50} of 1460 mg/kg bw, MON 52724 meets the criteria for USEPA Toxicity Category III.

G. Deficiencies: No deficiencies were identified.

Revised by the U.S. Environmental Protection Agency

STUDY TYPE: 28 day dietary toxicity study in rats

Report: Kirkpatrick, J. B. (2009c). A 28-day oral (diet) study of MON 52724 in rats. WIL Research Laboratories, LLC, unpublished report WI-09-161/WIL-50369. Sponsor: Monsanto Company, St. Louis, MO. MRID 47899506

Dates of Work: April 14, 2009 - May 2, 2009

Guidelines: OECD 407, EPA OPPTS 870.3050
Deviations: None.
PMRA DACO 4.5.4

GLP: Yes

EXECUTIVE SUMMARY: In a 28 day dietary toxicity test (MRID 47899506) groups of 10 rats/sex/group were exposed to DCGA (MON 52724) (Purity 98.1% Lot/batch No GLP-0904-19809-T) at dietary concentrations of 0, 500, 3000, 6000,or 12000 ppm. The average test substance consumption over the entire study was 0, 40, 240, 474, and 956 mg/kg/day for males and 0, 45, 265, 519, and 1063 mg/kg/day for females. All animals were observed twice daily for moribundity and mortality, clinical examinations were performed daily, and individual body weights were recorded weekly. Food consumption, functional observational battery (FOB) and motor activity were recorded twice weekly.

All animals survived to the scheduled necropsy. There were no adverse test substance related clinical observations, effects on organ weights or histological, or macroscopic findings. FOB and and motor activity were unaffected by treatment. Body weights were decreased 9% in males and 6% in females (not statistically significant).

The NOAEL was 474 mg/kg/day and the LOAEL was 956 mg/kg/day based upon decreased body weight in males. This study is classified totally reliable (acceptable/guideline), and it satisfies the guideline requirement for a 28-day oral toxicity study in rodents (OECD 407, OPPTS 870.3050).

I. MATERIALS AND METHODS:

DCGA was administered via the diet to 10 male and 10 female Sprague-Dawley rats per group for 28 consecutive days at dietary concentrations of 0, 500, 3000, 6000, and 12,000 ppm. The average test substance consumption over the entire study was 0, 40, 240, 474, and 956 mg/kg/day for males and 0, 45, 265, 519, and 1063 mg/kg/day for females. All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily and detailed physical examinations were performed weekly. Individual body weights and food consumption were recorded weekly. Functional observational battery and locomotor activity data were recorded for all animals prior to the initiation of dose administration and during study week 4. Clinical pathology evaluations (hematology, serum chemistry and urinalysis) were performed on all animals during study week 4. Complete necropsies were conducted on all animals, and selected organs were weighed at the scheduled necropsy. Standard tissues from all control and high-dose animals were stained with H&E and examined microscopically; perfusion of nervous tissues was not performed.

Test material:	DCGA (MON 52724); Lot no. GLP-0904-19609-T
Description:	Off-white powder
Lot/batch #:	GLP-0904-19809-T
Purity:	98.1% a.i.
CAS # of TGAI:	18688-01-2
Structure:	Not given

Test animals:

Species:	Rat
Strain:	Sprauge Dawley
Age at study initiation:	30 days
Wt. at study initiation:	Males: 150-193 g; Females: 108-143 g
Source:	Charles River Laboratories, Inc., Raleigh, NC
Housing:	Individual stainless steel, wire mesh cages suspended above cage-board
Diet:	Certified Rodent LabDiet #5002
Water:	Reverse osmosis treated water (made on-site)
Environmental	Temperature: 71±5°F

conditions:	Humidity:	42.9%-66.6%%
	Air changes:	10/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	3 days	

Dose selection rationale: Dosage levels were selected after a 5-day pilot study was conducted. In this study, there was decreased food consumption at 12000 ppm.

Dosage preparation and analysis: Formulations were prepared weekly by mixing appropriate amounts of test substance with Certified Rodent LabDiet #5002 and were frozen until usage. The stability of the test substance in the vehicle of delivery was not evaluated prior to the start of the study. Homogeneity (top, middle, and bottom) was evaluated weekly. During the study, samples of the treated food were analyzed weekly for all dosage levels for concentration.

Results:
Homogeneity analysis: *85% - 115%*
Stability analysis: *5.00 – 25.0 ppm*

Concentration analysis: *No less than 90% concentration*
The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

Positive control data for neurotox evaluations

Positive control data for FOB and motor activity were submitted to demonstrate the ability of the testing lab to detect neurotoxic effects. Decreased motor activity was tested with halperidol injection (study number WIL-99435) and increased motor activity was tested with nicotine and amphetamine injections (study number: WIL-99441). In both studies, 20 young rats/sex/group were tested on postnatal days 13, 17, 21, and 61. The test system was validated in both studies and decreased and increased motor activity was successfully detected.

Functional observational battery testing and motor activity in 6-week old rats were tested following treatments with chlorpyrifos at 3 different dose levels (study number WIL-99443). The test system was validated: home cage and open field assessments detected repetitive mouth movements, increased time until initiation of motor behavior, impaired gait, hunched body posture, splayed or dragging limbs, and decreased number of rears; neuromuscular assessment with a Rotarod detected impaired motor movement and/or balance and an increased apparent grip strength; physiological assessment detected increased catalepsy time and decreased body temperature. Decreased motor activity was also detected in this study.

I. RESULTS:

General observations:

All animals survived to the scheduled study termination date. There were no test substance-related clinical observations. There were no differences noted when the test substance treated males and females were compared to the control animals during functional observational battery and motor activity evaluations.

Body weights were decreased at study termination 9% in males and 6% in females, but were not statistically significant (Table 1). Body weights were comparable for all other treatment levels and controls. Food consumption was generally comparable between all test substance treated and control animals throughout the study although there was some indication of possible palatability issues at 12000 ppm during the first week of the study.

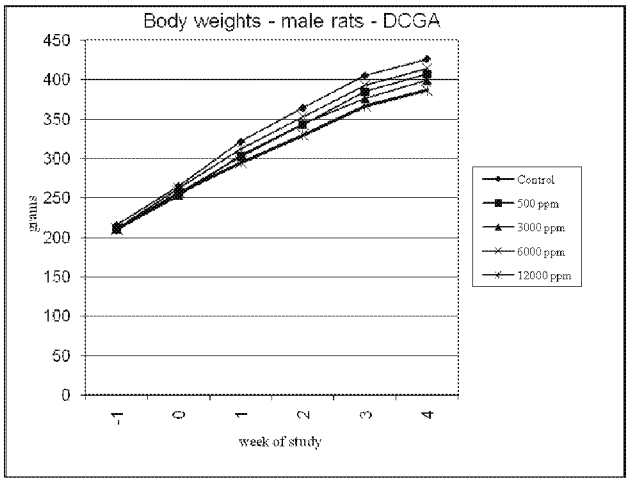
Table 1: Summary of Body Weights (g)

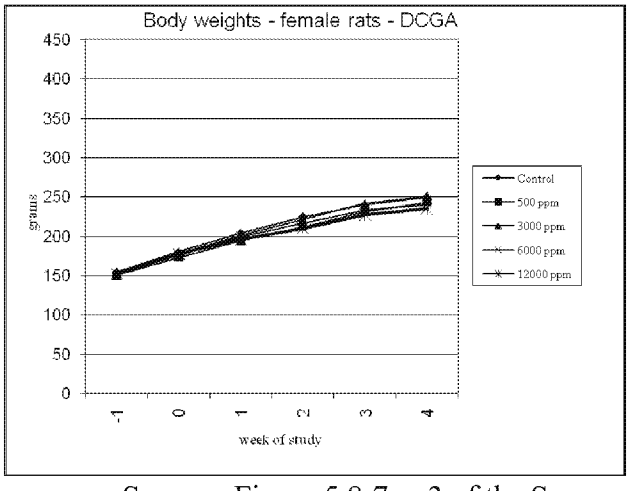
Summary of Body Weights (g±S.D.) in Males					
Dosage	Week 0 (start of study)	Week 1	Week 2	Week 3	Week 4
0 ppm	265±19.0	321±25.4	364±31.8	405±37.8	426±38.3
500 ppm	257±14.9	302±15.5	343±17.0	385±21.4	407±24.0
3000 ppm	253±12.9	304±19.9	344±24.2	376±30.9	399±34.9
6000 ppm	262±15.2	312±16.6	353±19.2	393±22.3	414±26.5
12000 ppm	257±21.4	295±26.5	330±33.6	367±41.1	387±44.5

Summary of Body Weights (g±S.D.) in Females					
Dosage	Week 0 (start of study)	Week 1	Week 2	Week 3	Week 4
0 ppm	180±11.5	204±13.6	225±14.3	240±17.1	250±21.1
500 ppm	173±10.7	194±12.6	212±15.8	231±17.0	243±19.7
3000 ppm	178±13.4	201±16.4	222±18.6	242±20.8	251±21.8
6000 ppm	175±16.2	199±22.3	217±24.2	233±27.8	240±31.4
12000 ppm	177±8.3	196±8.0	210±11.0	227±14.1	235±12.4

Source: Table 4, pp 79-82 of the Report

Figure 1: Body weights for male and female rats receiving DCGA in the diet for 28 days.





Source: Figure 5.8-7, p 3 of the Summary

Hematology, clinical chemistry, urinalysis:

The mean white blood cell counts were decreased in 12000 ppm males due to a decrease in absolute lymphocyte counts, but were within the range of historical control group means for the test laboratory (Table 5.8-16). There were no other hematology findings for any of the other test substance treated groups.

Table 2: Selected hematology parameters for DCGA in a 28-day rat study (mean±SD)

	0 ppm	500 ppm	3000 ppm	6000 ppm	12000 ppm	HC mean	HC mean range
Males							
WBC ($10^3/\mu L$)	11.6±3.21	11.9±3.21	9.8±1.92	10.3±3.28	8.2±2.17*	10.4±2.61	7.0-16.9
Lymphocytes ($10^3/\mu L$)	10.0±2.95	10.0±2.73	8.1±1.57	8.7±3.22	6.8±1.98*	8.6±2.26	5.6-13.9
Lymphocytes (%)	85.9±2.89	83.5±4.14	83.4±3.61	83.9±5.66	81.1±5.96	83.0±4.10	75.8-90.0
Females							
WBC ($10^3/\mu L$)	8.3±2.18	7.6±2.44	8.1±2.41	7.6±3.00	6.7±0.89	8.3±2.38	4.8-14.0
Lymphocytes ($10^3/\mu L$)	7.3±2.16	6.7±2.21	6.8±1.86	6.7±2.86	5.8±0.80	6.9±2.12	3.9-11.8
Lymphocytes (%)	87.3±4.09	87.0±4.39	84.4±6.07	87.1±2.81	86.9±3.36	83.4±4.92	75.6-91.2

HC = Historical control, WBC = white blood cells
N = 10/sex/dose for 28-day study data except 3000 ppm females where N = 9.
N = 669 for males and 674 for females for HC data.
* = $p \leq 0.05$.
Source: Table 5.8-16, p 4 of the Summary

Clinical chemistry evaluations showed a decrease in phosphorous levels in 12000 ppm males but not females and a decrease in urea nitrogen levels in 12000 ppm females but not males compared to concurrent controls (Table 5.8-17). The phosphorous levels were within the range of historical control means for the test laboratory and were considered non-adverse; decreased (rather than increased) urea nitrogen levels, especially in the absence of any other relevant toxicity, are considered toxicologically irrelevant. No other findings were observed for hematology and clinical chemistry evaluations in the study for any of the test substance groups.

Table 3: Selected serum chemistry parameters for DCGA in a 28-day rat study (means±SD)

	0 ppm	500 ppm	3000 ppm	6000 ppm	12000 ppm	HC mean	HC mean range
Males							
Phosphorus (mg/dL)	8.2±0.64	8.0±0.71	8.2±0.49	7.7±0.85	7.3±0.61*	9.4±1.20	7.2-13.5
Urea nitrogen (mg/dL)	14.7±2.90	14.9±1.20	13.8±2.44	12.8±0.99	13.1±1.12	13.8±2.38	8.9-17.5
Females							
Phosphorus (mg/dL)	7.3±1.06	7.9±1.11	7.3±0.86	7.7±1.47	7.2±0.85	8.3±1.13	5.4-13.0
Urea nitrogen (mg/dL)	15.1±1.13	16.2±1.32	15.4±1.46	14.5±2.26	13.1±1.81*	15.9±2.49	11.2-20.1

HC = historical control.
N = 10/sex/dose for 28-day study data.
N = 751 for males and 707 for females for phosphorus HC data; N = 761 for males and 716 for females for urea nitrogen HC data.
* = $p \leq 0.05$. Source: Table 5.8-17, p 4 of the Summary

Urinalysis evaluations showed decreased pH values for 12000 ppm males ($p<0.01$) and for 6000 and 12000 ppm females ($p<0.05$) compared to concurrent controls (Table 5.8-18). However, the mean pH values were within the range of group means for the WIL historical controls and were considered non-adverse although potentially test substance related effects.

Table 4: Selected urinalysis parameters for DCGA in a 28-day rat study (means±SD)

	0 ppm	500 ppm	3000 ppm	6000 ppm	12000 ppm	HC mean	HC mean range
Males							
pH	6.6±0.37	6.6±0.39	6.4±0.34	6.3±0.26	6.0±0.16**	6.6±0.48	5.8-7.8
Females							
pH	6.2±0.24	6.2±0.24	6.1±0.28	5.9±0.24*	5.9±0.24*	6.3±0.55	5.4-7.74

HC = historical control.
N = 10/sex/dose for 28-day study data. N = 493 for males and 478 for females for HC data.
* = $p \leq 0.05$; ** = $p \leq 0.01$. Source: Table 5.8-18, p 5 of the Summary

Gross pathology:

There were no adverse macroscopic findings at necropsy that were considered associated with test substance administration.

Organ weights:

For 12000 ppm males, relative brain, kidney, and testes weights were increased compared to control animal values (Table 5.8-19). Additionally, testes weights relative to fasted final body weights were significantly increased at 3000 ppm. There were no changes in absolute organ weights or corresponding histological findings. For females, absolute thyroid/parathyroid weights were lower for 500, 6000, and 12000 ppm groups and thyroid/parathyroid weights relative to brain weights were lower for 6000 and 12000 ppm groups compared to controls. However, there was no dose response and no microscopic correlates. There were no histological correlates for any of the body weights and they were not considered adverse.

Table 5. Selected organ weight data from a 28-day oral toxicity study in rats with DCGA (means±SD).

Dose level (ppm)	0	500	3000	6000	12000
	Males				
Final body weight (BW) (g)	396±34.3	379±22.9	371±31.4	383±21.6	357±39.3
Brain (g/100 g BW)	0.53±0.04	0.55±0.04	0.56±0.04	0.53±0.03	0.58±0.06*
Kidney (g/100 g BW)	0.89±0.07	0.90±0.06	0.92±0.07	0.90±0.05	0.97±0.07*
Testes (g/100 g BW)	0.79±0.07	0.85±0.06	0.88±0.05*	0.83±0.08	0.89±0.09**
	Females				
Final body weight (BW) (g)	232±18.8	224±17.9	231±22.5	222±27.6	215±12.4
Thyroid/parathyroid (g) – absolute wt.	0.015±0.002	0.013±0.002*	0.015±0.002	0.012±0.002**	0.013±0.002*
Thyroid/parathyroid (g/100 g BW)	0.007±0.001	0.006±0.001	0.006±0.001	0.005±0.001**	0.006±0.001
Thyroid/parathyroid (g/100 g brain wt.)	0.816±0.080	0.737±0.096	0.775±0.134	0.655±0.100**	0.697±0.092*

N = 10/sex/dose.
* = $p \leq 0.05$; ** = $p \leq 0.01$.
Source: Table 5.8-19, p 5 of the Summary

Histopathology:

There were no test substance related histological changes observed in the study. Minimal unilateral retinal dysplasia was found in 2 males from the 12,000 ppm group and in 3 control group females and was therefore not considered test substance-related.

Investigator's Conclusions:

Administration of DCGA to rats at dietary dose levels of 0, 500, 3000, 6000, and 12000 ppm for 28 days resulted in slightly (approximately 10% for males and 7% for females) but not statistically significantly decreased body weights at 12,000 ppm (approximately 956/1063 mg/kg/day, M/F), the highest dose tested. The NOAEL for DCGA for this study, therefore, is 6000 ppm (approximately 474/519 mg/kg/day, M/F).

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:

Reliability Rating: Totally reliable (acceptable/guideline)
This study is fully compliant with OECD 407

C. CONCLUSIONS:

Administration of DCGA to rats at dietary dose levels of 0, 500, 3000, 6000, and 12000 ppm for 28 days resulted in slightly (approximately 9% for males and 6% for females), but not statistically significantly, decreased body weights at 12,000 ppm (approximately 956/1063 mg/kg/day, M/F), the highest dose tested. There were also decreases in white blood cell counts and mean absolute lymphocyte counts in 12000 ppm males. The NOAEL was 474 mg/kg/day and the LOAEL was 956 mg/kg/day based upon decreased body weight.

Deficiencies: There were no deficiencies for this study.

Revised by U.S. Environmental Protection Agency

Study Type: 90-Day Oral (Diet) Study of MON 52708 in Rats

Report: IIA 5.8/6. Kirkpatrick, J. B. (2009a). A 90-Day Oral (Diet) Study of MON 52708 in Rats. WIL Research Laboratories, LLC, Ashland, OH, unpublished report WI-2006-014/WIL-50306, MRID 47899507.

Dates of Work: May 9, 2006 – February 7, 2008

Guidelines: OECD 408
EPA OPPTS 870.3100
Deviations: None.
PMRA DACO 4.3.1

GLP: Yes

EXECUTIVE SUMMARY:

In a 90 day dietary study (MRID 47899507), Sprague-Dawley (Crl:CD®[SD]) (10 rats/ sex/group) were exposed to DCSA (MON 52708, purity 97.9%; Lot/batchGLP-0603-16958-T) for 90-days. Final dietary concentrations were 500, 3000, 6000 and 12000 ppm. Due to potential problems with palatability observed in a previous rangefinding study, rats in the higher dose groups received slowly increasing doses during the first 1 to 2 weeks. Group 4 rats received the 3000 ppm diet during week 0 and the 6000 ppm diet during weeks 1 through 12. Group 5 rats received the 3000 ppm diet during week 0, the 6000 ppm diet during week 1 and the 12000 ppm diet during weeks 2 through 12.) The control group (Group 1) received the basal diet only throughout the study. The average test substance consumption over the entire study was 0, 32, 195, 362, or 659 mg/kg/day for males and 0, 37, 222, 436, or 719 mg/kg/day for females.

All animals were observed twice daily for mortality and morbidity. Clinical observations were made daily and detailed physical exams conducted weekly. Body weights and food consumption were measured weekly. Functional observational battery (FOB), locomotor activity and ophthalmic examination data were recorded prior to beginning exposure to MON 52708 and at the end of the study (week 12). Hematology, serum chemistry and urinalysis assessments were conducted during study week 13. Complete necropsies were conducted on all animals at study week 13. Selected organs were weighed at necropsy and selected tissues from all animals were examined microscopically.

Lower body weights were noted in the 12000 ppm group males and females throughout the study after dose ramping was concluded and final dosing levels were achieved (end of study week 2). Terminal mean body weights for the 12000 ppm males and females were 28.1% and 29.7% lower than controls, respectively. Body weights and food consumption in the 6000 ppm group females were also statistically significantly lower compared to

controls during the first few weeks of the study after ramping was concluded and generally remained lower but were not statistically significantly different for the rest of the study.

Food consumption in 12000 ppm males and females was decreased from the end of week 2 until approximately midway through the study. After approximately week 7, food consumption was increased in 12000 ppm males compared to controls and in 12000 ppm females was comparable to controls.

In the functional observation battery, there were no treatment-related effects noted during home cage, handling, open field, sensory, neuromuscular, or physiological observations. For the motor activity assessment, ambulatory counts were increased in 12000 ppm males by 59% ($p<0.005$), compared to controls, during the first 15 minute interval. Ambulatory counts were increased for that group in 2 other intervals, but not with statistical significance.

Hematological effects were noted in the 12000 ppm group. Effects included decreased red blood cell count, haemoglobin, MCHC, and hematocrit, and were more pronounced in females than in males.

Liver enzymes, including alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase, were increased in the 12000 ppm group. Relative liver weights were higher in the 12000 ppm group compared to controls, but absolute liver weights were not statistically different. There were no microscopic findings in the liver.

Microscopic lesions included an increase of bone marrow depletion in the sternum of the 12000 ppm group males and 6000 and 12000 ppm group females and hyperplasia of the epithelium in the glandular stomach of 12000 ppm group males and females. There were also four erosions in the glandular stomach, one each in males from the 6000 and 12000 ppm groups and two in females from the 12000 ppm group.

The NOAEL is 362 mg/kg/day and the LOAEL is 659 mg/kg/day based on decreased body weight, increased motor activity, decreased hematological parameters, and increased liver enzymes.

This study is classified totally reliable (acceptable/guideline) and satisfies the guideline requirement for a 90-day feeding study in the rat (EPA OPPTS guideline 870.3100 and OCED guideline Section 408).

I. MATERIALS AND METHODS

A. Materials

1. **Test material:** DCSA (MON 52708);
   Description: White powder
   Lot/Batch#: GLP-0603-16958-T
   Purity: 97.9%
   CAS #: Not given
   Compound Stability: The test substance was stable at room temperature
   Structure: Not available

2. **Test animals**

Species:	Rats, males and females	
Strain:	Sprague-Dawley, Crl:CD® (SD)	
Age/ weight at study initiation:	Approximately 8 weeks old at the initiation of dose administration. Male: 212 – 286 g Females: 166 – 213 g	
Source:	Charles River Laboratories, Inc., Raleigh, NC	
Housing	Individually in clean, stainless steel, wired-mesh cages suspended above cage-board.	
Diet:	Certified Rodent Lab-Diet® 5002 (PMI Nutrition International, LLC), *ad libitum*. The feed batch was analyzed for contaminants. No unacceptable levels of contaminants were present.	
Water:	Reverse osmosis-purified (on site) drinking water, *ad libitum*	
Environmental conditions:	**Temperature:**	22°C ± 3°C
	Humidity:	50% ± 20%
	Air changes:	At least 10/hr
	Photoperiod:	12 hrs Dark /12hrs light
Acclimation period:	16 days	

3. **Dose selection rational:**

Dosage levels of the test substance were selected based on previous results from a 14-day dose range-finding study, WIL-50305 (Kirkpatrick, 2007). In this study, lower body weight gains and/or body weight losses were observed in the 6000 and 12,000 ppm group males and females. The 14-day study demonstrated lower food consumption at the higher dose levels compared with controls, assumed due to palatability with the test substance. Therefore, the test substance was added to the diet in an escalating regimen in the definitive study.

4. **Dose preparation and analysis**:

Formulations were prepared weekly by mixing appropriate amounts of test substance with Rodent LabDiet #5002 (meal) and were stored at room temperature. Prior to the start of the study, stability of the test substance was evaluated for a period of 6, 8, and 11 days at room temperature storage and 32 days of frozen storage. Homogeneity (top, middle, and bottom) was evaluated in the 500 and 12,000 ppm dosing formulations. Samples for concentration analysis were collected during study weeks 0, 3, 7 and 12 from each dosing formulation including the control.

B. Study Design:

DCSA (MON 52708) was administered via the diet to five groups of 10 male and 10 female Sprague-Dawley rats for 90 consecutive days (Table 1). Final dietary concentrations of DCSA were 0, 500, 3000, 6000 and 12,000 ppm. However, to avoid palatability issues, the animals in the two highest dose groups received diets containing 3000 ppm DCSA for the first week and 6000 ppm for the second week. Starting with the third week of the study, the high-dose animals received diets containing 12,000 ppm. The average test substance consumption over the entire study was 0, 32, 195, 362, and 659 mg/kg/day for males and 0, 37, 222, 436, and 719 mg/kg/day for females.

All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily and detailed physical examinations were performed weekly. Individual body weights and food consumption were recorded weekly. Functional observational battery and locomotor activity data were recorded for all animals prior to the initiation of dose administration and during study week 12. Ophthalmic examinations were performed during study weeks -2 and 12. Clinical pathology evaluations (haematology, serum chemistry and urinalysis) were performed on all animals during study week 13. Complete necropsies were conducted on all animals, and selected organs were weighed at the scheduled necropsy. Standard tissues were examined microscopically from all control and high-dose animals and selected tissues were examined microscopically from intermediate dose animals.

TABLE 1. Study design				
Group Number	Treatment	Dose Level (ppm) [a]	Number of Animals	
			Males	Females
1	Vehicle (Basal Diet)	0	10	10
2	MON 52708	500	10	10
3	MON 52708	3000	10	10
4	MON 52708	6000[b]	10	10
5	MON 52708	12,000[c]	10	10

a- Test substance formulations were adjusted by a factor of 1.02 to account for test substance purity
b- The dose level of Group 4 was 3000 ppm for study week 0 and 6000 for study weeks 1 through 12
c- The dose level for group 5 was 3000 ppm for study week 0, 6000 ppm for study week 1 and 12000 ppm for study weeks 2 through 12

C. Positive Control Results for Neurotoxicity:

Positive control results for neurotoxicology measurements are reported in Appendix F of the WIL-50306 report (page 1003). This appendix contains summaries of four validation studies conducted by WIL (WIL 99140, WIL 99149, WIL 99263, WIL 99310). Briefly, these were as follows:

The purpose of the WIL-99140 study was to determine the appropriate length of time for their locomotor activity measurements based on the requirement that rats should approach normal activity levels by the last 20% of the session. The increase in activity measured in this study was due to the increased activity level that occurs in animals in a novel environment.

The purpose of the WIL-99149 study was to demonstrate the sensitivity of the SDI-PAS system for detecting alterations in locomotor activity in rats. Two compounds known to alter motor activity in rats were used: d-Amphetamine sulfate treatment which elicits increases in motor activity, and chlorpromazine hydrochloride which decreases motor activity. Results of this study indicated that the SDI-PAS system was sufficiently sensitive to detect dose-related increases and decreases in locomotor activity.

The purpose of WIL-99263 was to train personnel and assess inter-observer reliability in performing Functional Observational Battery Assessments (FOB). This study involved the use of two positive control compounds (3.3'-Iminodipropionitril (IDPN) and Parathion). Data are presented showing the sensitivity of the assessment of effects of these compounds on neurological effects of the test substances and the reliability between observers.

The purpose of WIL-99310 was to train personnel and assess inter-observer reliability of reported FOB results for neurotoxicity studies. Three positive control compounds were used (3.3'-Iminodipropionitril (IDPN), Parathion and d-Amphetamine). Corn oil was used as a negative control. The performance of four of the observers was consistent and deemed acceptable for detecting neurotoxicity effects.

II. RESULTS AND DISCUSSION

General observations:

All animals survived to the scheduled study termination date. One 12000 ppm female had clinical findings of thinness, pale extremities, extremities cool-to-the-touch, pale body, and faeces smaller than normal. These findings correlated with body weight loss and decreased food consumption for this animal during study weeks 10-13. There were no other test substance-related clinical observations.

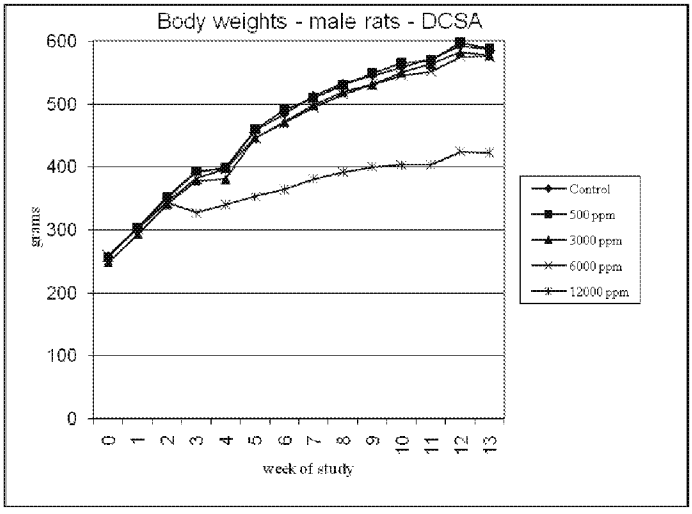
Body weights:

Lower body weights (Figure 1 and Table 2) were noted in the 12000 ppm group males and females throughout the study after dose ramping was concluded and final dosing levels were achieved (end of study week 2). Terminal mean body weights for the 12000 ppm males and females were 28.1% and 29.7% lower than controls, respectively.

Body weights and food consumption in the 6000 ppm group females were also statistically significantly lower compared to controls (<10%) during the first few weeks of the study after ramping was concluded and generally remained lower but were not statistically significantly different for the rest of the study.

Food consumption in 12000 ppm males and females was decreased from the end of week 2 until approximately midway through the study. After approximately week 7, food consumption was increased in 12000 ppm males compared to controls and in 12000 ppm females was comparable to controls.

Figure 1: Body weights for male and female rats receiving DCSA in the diet for 13 weeks.



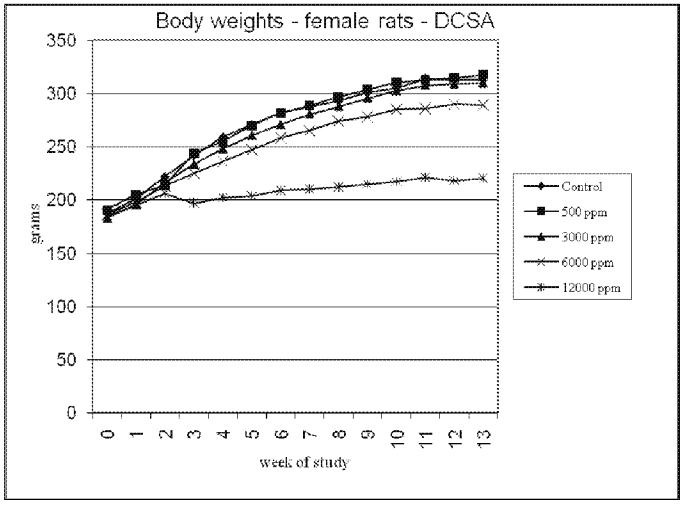


TABLE 2. Mean body weights (g) in Sprague-Dawley rats exposed daily to dietary doses of MON 52708 for 13 weeks.

TIME	0 PPM	500 PPM	3000 PPM	6000 PPM	12000 PPM
Males (n = 10) [a]					
Week -2	132±12.3	136±9.9	136±11.8	135±11.3	141±6.8
Week -1	163±9.0	163±8.7	161±8.4	162±8.3	163±8.8
Week 0	258±15.5	256±21.9	248±20.3	258±15.9	257±12.5
Week 1	303±18.8	303±29.4	293±26.7	302±18.9	302±14.5
Week 2	349±25.3	352±37.9	340±32.6	343±21.5	344±16.5
Week 3	392±29.3	392±42.7	378±35.9	382±24.1	327±23.1**
Week 4	397±24.7	398±38.7	380±40.2	396±33.8	340±28.3**
Week 5	458±37.4	460±50.7	446±41.3	446±32.6	353±36.7**
Week 6	484±37.1	491±53.7	471±43.5	470±36.8	364±40.6**
Week 7	513±48.3	510±59.1	498±45.4	495±42.3	381±44.5**
Week 8	532±47.0	530±62.1	519±46.8	515±46.0	392±47.4**
Week 9	545±48.9	549±63.5	531±49.6	531±48.7	400±50.9**
Week 10	558±51.0	565±66.5	550±49.8	545±51.9	407±53.5**
Week 11	571±57.7	569±63.7	563±52.3	552±55.5	403±48.2**
Week 12	592±63.4	598±68.5	582±55.0	575±55.1	424±55.8**
Week 13	587±61.5	588±65.0	577±52.4	576±58.7	422±55.3**
Females (n = 10) [a]					
WeeK -2	108±4.5	107±5.2	104±7.5	106±7.7	111±6.2
Week -1	131±7.2	130±7.2	130±7.2	131±8.4	131±7.1
Week 0	187±14.6	131±13.7	183±10.1	186±9.7	185±10.5
Week 1	201±15.8	205±14.1	196±10.4	198±13.8	195±12.4
Week 2	222±17.7	214±36.0	216±13.0	213±14.2	206±12.8
Week 3	242±23.8	244±17.3	234±16.4	225±14.1	197±12.1**
Week 4	259±27.7	255±21.9	248±18.5	236±15.8*	202±12.5**
Week 5	271±25.9	270±19.1	261±20.1	247±16.1*	204±12.5**
Week 6	282±25.8	282±20.9	271±24.3	258±16.7*	209±13.1**
Week 7	288±27.6	289±21.1	281±25.3	265±16.7	210±13.9**
Week 8	293±29.3	297±22.9	288±25.8	274±17.2	212±12.8**
Week 9	301±34.2	304±19.8	296±27.4	278±21.6	215±12.0**
Week 10	305±32.0	311±22.2	303±27.5	285±21.9	217±12.8**
Week 11	314±34.5	313±22.7	308±29.3	286±23.4	221±13.6**
Week 12	313±32.3	315±23.3	309±29.1	290±21.7	218±13.5**
Week 13	313±29.4	318±20.0	310±27.8	289±22.3	220±17.9**

[a] = Mean±SD

** = Body weight is significantly different from controls at 0.01 using Dunnett's test

Data were obtained from results in Tables 7and 8 on pages 88-95 of the study report

TABLE 3. Summary results of food consumption (g/kg/day) in Sprague-Dawley rats exposed daily to dietary doses of MON 52708 for 13 weeks.

TIME	0 PPM	500 PPM	3000 PPM	6000 PPM	12000 PPM
Males (n = 6-10) [a]					
WeeK -1-0	117±6.8	116±8.7	115±6.1	119±4.7	122±7.0
Week 0-1	97±4.2	96±3.6	94±4.4	94±3.2	98±4.1
Week 1-2	86±4.6	88±4.8	90±6.8	82±4.1	87±3.9
Week 2-3	80±4.0	80±4.0	79±2.9	77±5.5	59±10.6**
Week 3-4	80±7.8	80±5.1	82±6.2	75±6.6	69±6.5*
Week 4-5	61±5.6	61±6.4	62±5.3	64±5.6	60±6.9
Week 5-6	64±2.1	64±2.4	64±2.6	63±3.7	58±7.7*
Week 6-7	59±1.7	59±4.7	61±2.8	61±4.4	62±3.2
Week 7-8	55±0.7	57±2.6	56±2.7	58±3.3	59±3.7
Week 8-9	53±1.6	56±3.5	55±3.6	55±2.9	59±4.6**
Week 9-10	52±2.7	54±2.9	53±2.2	53±2.6	55±4.3
Week 10-11	51±0.8	53±2.0	53±1.7	53±2.5	58±4.3**
Week 11-12	51±2.1	51±2.4	51±2.2	51±2.4	57±3.1**
Week 12-13	45±5.8	45±3.6	45±3.7	47±5.2	51±3.6**
Females (n = 6-10) [a]					
WeeK -1-0	118±7.3	119±8.4	116±6.6	119±8.4	117±6.0
Week 0-1	100±6.1	97±5.2	96±5.9	99±10.7	99±9.1
Week 1-2	97±7.3	95±8.8	90±4.9	88±6.5*	85±3.3**
Week 2-3	89±3.6	79±27.5	87±7.6	82±3.7	66±16.8
Week 3-4	87±7.1	81±13.8	84±4.9	83±5.7	75±14.4
Week 4-5	83±6.3	83±3.6	80±4.3	81±4.0	71±7.3**
Week 5-6	76±3.7	77±3.3	74±3.8	76±4.6	66±5.6**
Week 6-7	70±4.8	71±2.4	72±4.0	75±5.4	59±9.0**
Week 7-8	66±7.1	67±4.1	66±4.5	71±4.8	60±6.0
Week 8-9	67±5.5	68±4.0	66±3.9	69±4.9	64±5.5
Week 9-10	66±4.7	64±4.2	63±4.5	69±7.4	62±5.0
Week 10-11	67±5.7	67±4.6	64±5.9	69±4.7	65±4.6
Week 11-12	67±9.5	64±3.6	62±4.3	69±13.3	65±6.5
Week 12-13	65±13.2	58±3.0	57±6.0	62±7.1	59±18.9

[a] = Mean±SD

* = Food consumption is significantly different from the control group at 0.05 using Dunnett's test

** = Food consumption is significantly different from the control group at 0.01 using Dunnett's test

Data were obtained from results in Tables 15 and 16 on pages 114 -119 of the study report

Functional Observation Battery

There were no treatment-related effects noted during home cage, handling, open field, sensory, neuromuscular, or physiological observations, other than decreased body weights in 12000 ppm males and females.

Motor Activity

Ambulatory counts were increased in 12000 ppm males by 59% ($p<0.005$), compared to controls, during the first 15 minute interval. Ambulatory counts were increased for that group in 2 other intervals, but not with statistical significance.

Haematology, clinical chemistry, urinalysis:

Test substance-related haematology effects consisted of lower red blood cell mass as indicated by red blood cell count, haemoglobin, MCHC, and hematocrit. These effects were more notable in females than in males: Hemoglobin was decreased only 8% in 12000 ppm males, was decreased 8% in 6000 ppm females, and was decreased 28% in 12000 ppm females.

Platelet counts were slightly decreased in 12000 ppm rats, however, clotting time (APTT) was decreased in 12000 ppm rats, which is not ordinarily considered toxicologically adverse.

Table 4: Selected haematology parameters for DCSA (MON 52708) in a 90-day rat study (mean±SD)

	0 ppm	500 ppm	3000 ppm	6000 ppm	12000 ppm
Males					
RBC (10^6/µL)	9.07±0.37	9.19±0.38	9.37±0.36	8.88±0.76	8.35±0.42**
HGB (g/dL)	16.7±0.37	16.3±0.63	16.9±0.49	16.4±1.16	15.3±0.64**
HCT (%)	48.1±1.41	47.4±2.19	49.3±1.87	47.7±3.33	45.4±2.04*
MCHC (g/dL)	34.8±0.48	34.5±0.58	34.4±0.52	34.4±0.37	33.8±0.50**
Platelet (10^3/µL)	979±182	973±99	899±88	825±190	742±120**
APTT (seconds)	21.0±1.3	21.9±1.5	20.7±1.2	19.9±1.7	18.0±1.7**
Females					
RBC (10^6/µL)	8.50±0.30	8.23±0.39	8.28±0.45	7.78±0.80	6.42±1.85**
HGB (g/dL)	16.3±0.57	15.8±0.44	16.0±0.76	15.0±1.38	11.7±3.26**
HCT (%)	47.0±1.83	44.5±2.08	45.1±1.79	43.0±4.46	34.9±9.37**
MCHC (g/dL)	34.7±0.47	35.6±1.27	35.5±0.37	34.9±0.86	33.2±1.29**
Platelet (10^3/µL)	775±150	1080±153**	868±140	718±129	862±149
APTT (seconds)	17.8±3.0	17.6±1.9	17.6±2.4	17.9±2.3	15.0±2.4*

N = 10/sex/dose. * = $p \leq 0.05$; ** = $p \leq 0.01$.
RBC = red blood cells, HGB = haemoglobin, HCT = hematocrit, MCHC = mean corpuscular haemoglobin concentration, APTT = activated partial thromboplastin time

Test substance-related serum chemistry effects are shown in Table 5. In both sexes, slightly higher alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase values occurred at the 12000 ppm dose level.

Decreased globulins resulted in lower total protein and alterations in A/G ratio in 12000 ppm males and in 6000 and 12000 ppm females compared to controls. Albumin was also lower in the 12000 ppm group females.

There were variable increases in BUN and creatinine when males were compared to females: creatinine was higher compared to controls for males at 6000 and 12000 ppm, while urea nitrogen was higher in females at the 12000 ppm dose. In neither case, were there corroborating microscopic lesions.

The only urinalysis effect was lower pH in the 12000 ppm group males.

Table 5: Selected serum chemistry parameters for DCSA (MON 52708) in a 90-day rat study (means±SD)

	0 ppm	500 ppm	3000 ppm	6000 ppm	12000 ppm
Males					
Albumin (g/dL)	4.3±0.20	4.3±0.16	4.3±0.19	4.3±0.13	4.2±0.17
Total protein (g/dL)	7.1±0.20	7.3±0.15	7.1±0.32	7.0±0.23	6.2±0.34**
Globulin (g/dL)	2.8±0.25	3.0±0.16	2.9±0.18	2.7±0.10	2.0±0.26**
A/G ratio	1.55±0.18	1.48±0.13	1.51±0.08	1.56±0.05	2.13±0.27**
Urea nitrogen (mg/dL)	14.2±1.7	13.5±1.7	14.3±1.8	13.6±1.5	15.7±2.6
Creatinine (mg/dL)	0.2±0.05	0.2±0.07	0.3±0.05	0.4±0.07**	0.4±0.07**
ALP (U/L)	91±18.8	90±21.0	87±14.5	89±23.4	142±27.4**
ALT (U/L)	45±13.7	40±9.4	48±11.6	39±6.4	66±31.2*
AST (U/L)	90±20.1	82±18.2	88±11.4	87±10.7	135±66.6*
Glucose (mg/dL)	119±11.9	114±12.0	114±10.7	109±10.2	99±7.3**
Females					
Albumin (g/dL)	4.7±0.42	4.9±0.24	5.0±0.35	4.6±0.21	4.4±0.25*
Total protein (g/dL)	7.3±0.41	7.4±0.24	7.6±0.45	6.7±0.35*	5.9±0.50**
Globulin (g/dL)	2.6±0.21	2.5±0.21	2.6±0.22	2.1±0.18**	1.5±0.28**
A/G ratio	1.85±0.26	1.93±0.23	1.95±0.19	2.21±0.19*	2.97±0.52**
Urea nitrogen (mg/dL)	15.4±1.5	15.8±1.9	16.5±2.3	19.3±6.3	23.7±7.9**
Creatinine (mg/dL)	0.4±0.05	0.3±0.05	0.3±0.03	0.4±0.07	0.4±0.11
ALP (U/L)	56±12.8	51±15.3	54±10.3	57±19.2	98±38.6**
ALT (U/L)	36±13.0	40±17.7	41±16.8	35±6.7	82±122.7
AST (U/L)	79±9.4	93±17.5	81±15.2	85±10.4	127±83.5*
Glucose (mg/dL)	108±7.0	116±11.1	114±8.4	109±13.2	102±9.3

N = 10/sex/dose.
* = $p \leq 0.05$; ** = $p \leq 0.01$.
A/G ration = albumin to globulin ratio, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase

Gross pathology, organ weights, and histopathology:

Liver weights relative to final body weights were higher ($p<0.01$) in the 12000 ppm group males and females compared to controls but absolute liver weights were not statistically different (Table 6) and there were no microscopic findings in the liver.

A macroscopic finding of pale discoloration was observed in several organs (adrenal gland, kidneys, pancreas, mandibular salivary gland, and whole body) in the 12000 ppm group females.

Table 6: Liver weights at terminal sacrifice (week 13) for DCSA (MON 52708) in a 90-day rat study (means±SD)

	Males					Females				
	0 ppm	**500 ppm**	**3000 ppm**	**6000 ppm**	**12000 ppm**	**0 ppm**	**500 ppm**	**3000 ppm**	**6000 ppm**	**12000 ppm**
Absolute liver weight (g)	15.47 ±2.26	15.53 ± 2.19	14.90 ± 1.80	14.47 ± 1.77	13.59 ± 1.73	7.86± 0.82	7.98± 0.72	7.55± 0.89	7.57± 0.44	7.54± 0.82
Relative (to body) liver weight (g)	2.73± 0.21	2.74± 0.17	2.67± 0.19	2.62± 0.18	3.34± 0.12**	2.71± 0.17	2.69± 0.13	2.62± 0.29	2.80± 0.24	3.70± 0.16**

** = $p \leq 0.01$
N = 10 for all dose groups.

Microscopically, there was an increase of bone marrow depletion in the sternum of the 12000 ppm group (Table 7). Marrow depletion is a term used to characterize replacement of haematopoietic tissue with mature adipose tissue.

Administration of the test substance was associated with hyperplasia of the epithelium in the fundus and pylorus of the glandular stomach of 12000 ppm group males and females (Table 7). Mucosal epithelial hyperplasia was not present at any other dose level. Four erosions were observed in the glandular stomach, one each in males from the 6000 and 12000 ppm groups and two in females from the 12000 ppm group. The severity of the erosions was moderate in one female and minimal in the other female and in both males. The erosions did not occur in areas of mucosal epithelial hyperplasia.

Table 7: Incidence of bone marrow depletion and stomach hyperplasia and erosion in male and female rats fed DCSA in the diet for 90 days

	Males					Females				
	0 ppm	500 ppm	3000 ppm	6000 ppm	12000 ppm	0 ppm	500 ppm	3000 ppm	6000 ppm	12000 ppm
Bone marrow, sternum – examined	10	10	10	10	10	9	10	10	10	10
Depletion, Generalized	0	0	0	0	5	0	0	0	1	2
Minimal	0	0	0	0	1	0	0	0	1	1
Mild	0	0	0	0	4	0	0	0	0	1
Stomach, glandular – examined	10	10	10	10	10	10	10	10	9	10
Hyperplasia, Epithelial	0	0	0	0	4	0	0	0	0	9
Minimal	0	0	0	0	3	0	0	0	0	3
Mild	0	0	0	0	1	0	0	0	0	4
Moderate	0	0	0	0	0	0	0	0	0	2
Erosion	0	0	0	1	1	0	0	0	0	2
Minimal	0	0	0	1	1	0	0	0	0	1
Mild	0	0	0	0	0	0	0	0	0	0
Moderate	0	0	0	0	0	0	0	0	0	1

III. EVALUATION, SUMMARY AND CONCLUSIONS BY REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Programs/U.S. EPA

B. REVIEWER'S COMMENTS:

RELIABILITY RATING: Reliable (Acceptable/Guideline).
This study is fully compliant with OECD.408.

Effects of toxicological significance found in 12000 ppm males and females included decreased body weight, decreased hematological parameters, and elevated liver enzymes. The registrant assigned a NOAEL of 3000 ppm in females because of depressed body weight midway through the study and slight effects upon serum globulins and bone marrow depletion in the sternum. However, these minor effects are not considered toxicologically adverse and the NOAEL for both males and females is 6000 ppm.

Deficiencies: No deficiencies were noted in this study.

Revised by U.S. Environmental Protection Agency

Study Type: A 90-Day Oral (Capsule) Toxicity Study of MON 52708 in Beagle Dogs

Report: IIA 5.8/7. Kirkpatrick, J. B. (2011). Amended Report: A 90-day oral (capsule) toxicity study of MON 52708 in Beagle dogs. WIL Research Laboratories, LLC, unpublished report WI-2007-032/WIL-50335, MRID 48358002.

Dates of Work: April 26, 2007 – September 11 2009

Guidelines: OECD 409, EPA OPPTS 870.3150
Deviations: None.
PMRA DACO 4.3.2

GLP: Yes

EXECUTIVE SUMMARY:

In a 90 day oral capsule study (MRID 48358002), Beagle dogs (5 animals/sex/group) were treated with DCSA (MON 52708 purity 97.7%; Lot/batchGLP-0603-16958-T) for 90 days with doses of 0, 15, 50 and 150 mg/kg/day.

All animals were observed twice daily for mortality and morbidity. Clinical observations were performed daily and detailed physical exams were conducted weekly. Body weights were measured weekly. Food consumption was recorded daily and reported weekly. Clinical pathology evaluations included hematology, coagulation, serum chemistry and urinalysis and were conducted prior to initiation of dosing and during study weeks 6 and 13. Ophthalmic examinations were conducted prior to initiation of dosing and during study week 12. Complete necropsies were conducted on all animals during study week 13. Selected organs were weighed at necropsy and selected tissues were examined microscopically.

One female in the 150 mg/kg/day dose group was euthanized in extremis on day 50 of the study. Death was associated with repeated emesis, electrolyte imbalance, and severe dehydration. All other animals survived to the scheduled necropsy.

Statistically significant decreases were observed in cumulative body weight gains in both males and females in the 150 mg/kg/day groups. Absolute mean body weights in these groups were about 11% lower than controls at the end of the study, though the differences were not statistically significant. Decreased food consumption was observed in females in the 150 mg/kg/day group during study weeks 1 -2 and 3-4. Male food consumption was not different from controls. Abnormal excreta and emesis were present in the 150 mg/kg/day male and female groups. Abnormal excreta began on study day 0; emesis began on study day 2. Both effects persisted to the end of the study.

Coagulation effects were observed in both males and females: APTT values were higher in males in the 150 mg/kg/day at study week 13 and in females in the 150 mg/kg/day group at study week 6.

Liver weights relative to body weights were higher in males and females in the 150 mg/kg/day groups. Hypertrophy of periportal hepatocytes was observed in the livers of both sexes in the 150 mg/kg/day groups.

The NOAEL is 50 mg/kg/day and the LOAEL is 150 mg/kg/day based on mortality, decreased body weight, clinical signs (abnormal excreta and emesis), and increased clotting time.

This study is classified totally reliable (acceptable/guideline) and satisfies the guideline requirement (EPA OPPTS guideline 870.3150 and OCED guideline Section 409) for a 90-day dog study.

I. MATERIALS AND METHODS

A. Materials

1. **Test material:**	DCSA (MON 52708);
Description:	White powder
Lot/Batch#:	GLP-0603-16958-T
Purity:	97.7%
CAS #:	None given
Compound Stability:	Not determined in the study; Considered to be stable at room temperature
Structure:	Not available

2. Test animals

Species:		Dogs, males and females
Strain:		Beagle
Age/ weight at study initiation:		Approximately 4.5 months old at the initiation of dose administration. Male: 5.9 – 9.1 kg Females: 4.7 – 8.2 kg
Source:		Ridgelan Farms, Inc., Mt Horeb, Wisconsin
Housing		Individually in stainless steel cages elevated above stainless steel cage pans
Diet:		Certified Canin Lab-Diet® 5007 (PMI Nutrition International, LLC), *ad libitum*. The feed batch was analyzed for contaminants. No unacceptable levels of contaminants were present.
Water:		Reverse osmosis-purified (on site) drinking water, *ad libitum*
Environmental conditions:	**Temperature:**	20°C ± 3°C
	Humidity:	50% ± 20%
	Air changes:	At least 10/hr
	Photoperiod:	12 hrs Dark /12hrs light
Acclimation period:		12 days

3. Dose Selection Rational:

Information on the rational for dose selection was not provided in this study.

4. Dose Preparation and Analysis:

Certificates of analysis were provided by the sponsor. The appropriate amounts of test substance were weighed and placed into gelatin capsules. One capsule containing the test substance was dispensed daily for each dog in the 15, 50, and 150 mg/kg/day groups. Dogs in the control group received empty capsules (1 capsule/dog/day). Documentation regarding stability, purity and identity of the test substance are on file with the sponsor. The test substance was stored at room temperature and was considered stable under this condition

B. Study Design:

In life dates: 26 April 2007 - 28 November 2007

DCSA (MON 52708) was administered in capsules once daily, for a minimum of 90 consecutive days, to 3 groups of Beagle dogs at dosage levels of 0, 15, 50, and 150 mg/kg/day, shown in Table 1. The control and treatment groups each consisted of 5 animals/sex. All animals were scheduled for necropsy at the end of the 90-day treatment period.

TABLE 1. Study design

Group Number	Treatment	Dose Level (mg/kg/day)	Number of Animals	
			Males	Females
1	Empty capsule	0	5	5
2	MON 52708	15	5	5
3	MON 52708	50	5	5
4	MON 52708	150	5	5

The animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed weekly. Individual body weights were recorded weekly. Food consumption was recorded daily and reported weekly. Clinical pathology evaluations (haematology, coagulation, serum chemistry, and urinalysis) were performed prior to the initiation of dose administration and during study weeks 6 and 13. Ophthalmic examinations were performed during study weeks -1 and 12.

Complete necropsies were performed on all animals, and selected organs were weighed at the scheduled necropsy. Standard tissues from all control and high-dose animals were examined microscopically. In addition, liver tissue was examined microscopically from all low and mid-dose animals.

II. RESULTS AND DISCUSSION

General observations:

One female in the 150 mg/kg/day dose group was euthanized in extremis on day 50 of the study. Death was associated with repeated emesis, electrolyte imbalance, and severe dehydration. All other animals survived to the scheduled necropsy.

Clinical observations included some instances of abnormal excreta (diarrhea, soft feces and/or mucoid feces) and/or emesis primarily in the 50 and 150 mg/kg/day test substance-treated groups. The observation at 50 mg/kg/day was judged to be slight (soft feces) and, therefore, non-adverse.

Cumulative mean body weight gains, shown in Table 2, were lower compared to controls for the 150 mg/kg/day group males and females throughout the study (33% and 42%, respectively, at study week 13). Mean absolute body weights (Table 3) in these males

and females were 10% and 12% lower than the controls, respectively, at the end of the study. Test substance-related reductions in food consumption (Table 4) were also noted for the 150 mg/kg/day group females throughout the study.

TABLE 2. Mean cumulative body weight changes (kg) in Beagle dogs orally exposed daily with capsules containing doses of MON 52708

Time	Daily Dose (mg/kg/day)							
	Male (n=5)				Female (n=5)			
	0	15	50	150	0	15	50	150
Week 0 to 1	0.4 ± 0.23[a]	0.4 ± 0.20	0.4 ± 0.37	0.1 ± 0.29	0.3 ± 0.17	0.4 ± 0.19	0.1 ± 0.25	0.0 ± 0.40
Week 0 to 2	0.9 ± 0.23	0.7 ± 0.46	1.0 ± 0.42	0.3 ± * 0.27	0.7 ± 0.18	0.8 ± 0.36	0.6 ± 0.37	0.0 ± * 0.47
Week 0 to 3	1.1 ± 0.26	1.1 ± 0.37	1.3 ± 0.41	0.6 ± * 0.19	0.9 ± 0.27	1.0 ± 0.32	0.9 ± 0.36	0.3 ± 0.51
Week 0 to 4	1.5 ± 0.28	1.5 ± 0.32	1.7 ± 0.36	0.9 ± * 0.11	1.2 ± 0.23	1.3 ± 0.49	1.2 ± 0.46	0.5 ± * 0.41
Week 0 to 5	1.7 ± 0.38	1.9 ± 0.53	2.1 ± 0.49	1.3 ± 0.19	1.5 ± 0.26	1.6 ± 0.46	1.4 ± 0.38	0.7 ± * 0.50
Week 0 to 6	2.1 ± 0.39	2.3 ± 0.52	2.4 ± 0.36	1.7 ± 0.31	1.9 ± 0.37	2.0 ± 0.70	1.9 ± 0.51	1.0 ± * 0.48
Week 0 to 7	2.5 ± 0.44	2.5 ± 0.55	2.7 ± 0.38	1.7 ± * 0.41	2.1 ± 0.35	2.1 ± 0.71	2.0 ± 0.48	1.0 ± * 0.72
Week 0 to 8	2.7 ± 0.47	3.0 ± 0.52	3.1 ± 0.48	2.0 ± 0.44	2.4 ± 0.59	2.6 ± 0.71	2.5 ± 0.45	1.2 ± * 0.90
Week 0 to 9	3.1 ± 0.46	3.4 ± 0.62	3.4 ± 0.45	2.4 ± 0.48	2.6 ± 0.44	2.8 ± 0.83	2.8 ± 0.58	1.3 ± * 0.86
Week 0 to 10	3.2 ± 0.54	3.6 ± 0.57	3.6 ± 0.59	2.4 ± 0.51	2.8 ± 0.50	2.9 ± 0.85	2.9 ± 0.60	1.5 ± 0.98
Week 0 to 11	3.4 ± 0.63	3.7 ± 0.63	3.9 ± 0.63	2.5 ± 0.39	2.8 ± 0.64	2.9± 0.86	3.1 ± 0.67	1.7 ± 0.82
Week 0 to 12	3.8 ± 0.58	4.2 ± 0.64	4.2 ± 0.68	2.6 ± * 0.54	3.2 ± 0.69	3.3 ± 0.96	3.4 ± 0.73	1.8 ± 0.99
Week 0 to 13	4.0 ± 0.81	4.2 ± 0.74	4.6 ± 0.73	2.7 ± * 0.63	3.3 ± 0.85	3.5 ± 0.95	3.6 ± 0.72	1.9 ± 0.83

[a] = Standard deviation

* = Body weight gain is significantly different from the same week control group at 0.05 using Dunnett's test

Data were obtained from results in Table 5 on pages 72-77 of the study report

TABLE 3. Mean body weights (kg) in Beagle dogs orally exposed daily with capsules containing doses of MON 52708.

Time	Daily Dose (mg/kg/day)							
	Male (n=5)				Female (n=5)			
	0	15	50	150	0	15	50	150
Week -2	7.3 ± 1.04[a]	7.5 ± 0.88	7.3 ± 7.3	7.4 ± 0.98	6.2 ± 0.91	6.0 ± 1.08	6.1 ± 1.32	6.0 ± 1.07
Week -1	7.5 ± 1.11	7.8 ± 0.94	7.5± 0.84	7.7± 0.96	6.4± 0.96	6.3 ± 1.11	6.3 ± 1.39	6.3 ± 1.14
Week 0	7.5 ± 1.11	7.8 ± 0.94	7.5 ± 0.84	7.7 ± 0.96	6.4 ± 0.96	6.3 ± 1.11	6.3 ± 1.39	6.3 ± 1.14
Week 1	7.9 ± 1.07	8.2 ± 1.11	8.0 ± 0.74	7.8 ± 1.12	6.8 ± 0.93	6.7 ± 0.97	6.5 ± 1.13	6.3 ± 1.15
Week 2	8.5 ± 1.22	8.5 ± 1.25	8.6 ± 0.81	8.0 ± 1.18	7.2 ± 0.99	7.1 ± 0.94	6.9 ± 1.22	6.3 ± 1.11
Week 3	8.7 ± 1.20	8.9 ± 1.27	8.8 ± 0.81	8.2 ± 0.94	7.3 ± 1.00	7.3 ± 0.93	7.2 ± 1.27	6.6 ± 1.21
Week 4	9.0 ± 1.22	9.3 ± 1.20	9.3 ± 0.93	8.6 ± 1.03	7.6 ± 0.95	7.6 ± 0.86	7.5 ± 1.36	6.8 ± 1.09
Week 5	9.3 ± 1.26	9.6 ± 1.31	9.6 ± 0.97	8.9 ± 0.99	8.0 ± 0.98	7.9 ± 0.92	7.7 ± 1.31	7.0 ± 1.17
Week 6	9.7 ± 1.22	10.0 ± 1.28	10.0 ± 0.98	9.3 ± 1.13	8.4 ± 0.98	8.4 ± 0.85	8.2 ± 1.19	7.3 ± 1.03
Week 7	10.0 ± 1.24	10.2 ± 1.25	10.3 ± 0.88	9.4 ± 1.12	8.6 ± 1.09	8.5 ± 0.72	8.4 ± 1.30	7.3 ± 1.34
Week 8	10.3 ± 1.32	10.8 ± 1.26	10.7 ± 1.10	9.7 ± 1.15	8.9 ± 1.26	8.9 ± 0.83	8.8 ± 1.27	7.8 ± 1.12
Week 9	10.6 ± 1.28	11.1 ± 1.32	11.0 ± 1.12	10.1 ± 1.17	9.1 ± 1.05	9.1 ± 0.97	9.1 ± 1.16	7.9 ± 1.12
Week 10	10.7 ± 1.36	11.4 ± 1.17	11.2 ± 1.17	10.1 ± 1.21	9.2 ± 1.13	9.2 ± 0.85	9.2 ± 1.19	8.1± 1.53
Week 11	10.9 ± 1.46	11.5 ± 1.20	11.4 ± 1.27	10.2 ± 1.03	9.2 ± 1.17	9.3 ± 0.91	9.4 ± 1.12	8.3 ± 1.28
Week 12	11.3± 1.41	11.9 ± 1.23	11.7 ± 1.28	10.3± 1.39	9.6 ± 1.28	9.6 ± 0.96	9.7 ± 1.10	8.4 ± 1.09
Week 13	11.5 ± 1.56	11.9 ± 1.01	12.1 ± 1.34	10.3 ± 1.39	9.7 ± 1.27	9.8 ± 1.04	9.9 ± 1.01	8.5 ± 0.95

[a] = Standard deviation

No statistically significantly differences were observed between exposed groups vs controls.

Data were obtained from results in Table 4 on pages 64-7 of the study report

TABLE 4. Mean Food Consumption (g/animal/day) in dogs orally exposed daily with capsules containing doses of MON 52708

Time	Daily Dose (mg/kg/day)							
	Male (n=5)				Female (n=5)			
	0	15	50	150	0	15	50	150
Week -1 to 0	296 ± 49.6[a]	327 ± 26.9	315 ± 50.9	316 ± 37.3	264 ± 34.8	281 ± 27.0	280 ± 39.9	254 ± 24.8
Week 0 to 1	309 ± 63.2	359 ± 37.5	334 ± 52.6	321 ± 74.5	288 ± 59.1	312 ± 41.6	291 ± 22.6	255 ± 67.5
Week 1 to 2	332 ± 63.0	369 ± 30.9	344 ± 46.8	321 ± 79.1	317 ± 36.8	327 ± 28.8	291± 33.3	250 ± * 44.1
Week 2 to 3	330 ± 63.5	378 ± 21.4	360 ± 47.7	358 ± 44.3	306 ± 41.0	313 ± 15.0	315 ± 26.9	266 ± 31.8
Week 3 to 4	341 ± 56.1	381 ± 27.0	372 ± 37.4	367 ± 49.7	330 ± 43.9	337 ± 35.2	341 ± 32.1	265 ± * 25.1
Week 4 to 5	336 ± 54.2	386 ± 16.9	373 ± 41.0	381 ± 35.0	330 ± 49.0	349 ± 38.4	336 ± 30.4	280 ± 19.9
Week 5 to 6	351 ± 54.4	385 ± 18.6	371 ± 43.1	384 ± 29.0	329 ± 34.7	347 ± 28.1	353 ± 22.3	289 ± 29.0
Week 6 to 7	344 ± 40.3	343 ± 24.8	361 ± 26.7	353 ± 59.0	310 ± 28.9	328 ± 27.8	332 ± 28.3	257 ± 54.6
Week 7 to 8	358 ± 50.6	391 ± 9.8	377 ± 33.2	379 ± 47.6	325 ± 42.1	345 ± 35.0	357 ± 50.8	277 ± 64.3
Week 8 to 9	349 ± 46.7	385 ± 22.3	379 ± 23.5	372 ± 48.4	320 ± 52.3	355 ± 45.7	365 ± 41.2	275 ± 50.7
Week 9 to 10	344 39.4	384 29.1	378 32.4	374 57.0	303 ± 46.1	347± 17.9	346 ± 43.5	283 ± 39.2
Week 10 to 11	352 38.0	374 26.9	373 44.6	375 49.0	324 ± 48.6	358 ± 25.3	350 ± 43.8	307 ± 17.1
Week 11 to 12	346 36.8	385 15.0	376 28.5	371 52.3	318 ± 55.4	341 ± 31.3	340 ± 32.8	262 ± 73.5
Week 12 to 13	316 54.2	343 49.9	350 41.7	352 67.5	271 ± 55.1	318 ± 33.6	307 ± 44.8	269 ± 15.8

[a] = Standard deviation
* = Body weight is significantly different from the same week control group at 0.05 using Dunnett's test
Data were obtained from results in Table 5 on pages 78-83 of the study report

Haematology, clinical chemistry, urinalysis:

Haematology and coagulation observations consisted of statistically significantly higher APTT in 50 and 150 mg/kg/day group males at study week 13 and in the 150 mg/kg/day group females at study week 6 (Table 5). The increased APTT in the 50 mg/kg/day group males at study week 13 was slight, approximated the WIL Laboratories historical control mean value (12.3±1.5, range 10.9-15.2, N=458), and was not considered toxicologically significant due to the small magnitude (<10%) of the change. All other haematology parameters evaluated were comparable for treated and control animals.

Table 5: Selected haematology parameters for DCSA (MON 52708) in a 90-day dog study (means±SD)

	0 mg/kg/day	15 mg/kg/day	50 mg/kg/day	150 mg/kg/day
Males				
APPT (seconds) – pre-test	11.7±0.6	11.5±0.4	12.0±0.5	11.5±0.4
APPT (seconds) – week 6	12.5±0.8	11.5±0.4	12.4±0.8	13.2±0.6
APTT (seconds) – week 13	11.6±0.6	11.1±0.5	12.6±0.5*	13.0±0.5**
Females				
APPT (seconds) – pre-test	11.5±0.6	11.6±0.3	12.0±0.5	11.8±0.7
APPT (seconds) – week 6	11.4±0.2	11.8±0.6	12.2±1.0	14.0±1.6**
APTT (seconds) – week 13	11.5±0.5	12.0±0.9	12.2±1.6	13.4±1.0

N = 5/sex/dose except 150 mg/kg/day females at week 13 where N = 4.
* = $p \leq 0.05$; ** = $p \leq 0.01$.
APTT = activated partial thromboplastin time

Several serum chemistry effects were noted, but were not considered toxicologically significant. These included lower alkaline phosphatase in the 150 mg/kg/day group males and females at study weeks 6 and 13; lower alanine aminotransferase in the 150 mg/kg/day group males at study week 13, and lower gamma glutamyltransferase in the 150 mg/kg/day group males and females at study week 13 (Table 6). Total protein in the 50 and 150 mg/kg/day group males at study week 13 was slightly (5 and 7%, respectively) but statistically significantly lower than control values, however, individual albumin and globulin levels were comparable to controls in these groups. (Table 6).

Table 6: Selected serum chemistry parameters at terminal sacrifice (week 13) for DCSA (MON 52708) in a 90-day dog study (means±SD)

	0 mg/kg/day	15 mg/kg/day	50 mg/kg/day	150 mg/kg/day
Males				
Albumin (g/dL)	3.5±0.12	3.5±0.08	3.4±0.13	3.3±0.16
Total protein (g/dL)	5.8±0.04	5.6±0.13	5.5±0.30*	5.4±0.11**
Globulin (g/dL)	2.3±0.16	2.1±0.15	2.1±0.21	2.1±0.11
A/G ratio	1.53±0.15	1.63±0.13	1.67±0.14	1.61±0.14
Urea nitrogen (mg/dL)	13.8±1.4	13.3±2.1	14.1±2.8	15.9±3.1
Creatinine (mg/dL)	0.5±0.09	0.6±0.09	0.6±0.09	0.5±0.13
ALP (U/L)	93±14.7	107±19.8	77±20.0	50±5.1**
ALT (U/L)	40±4.8	42±1.9	37±8.3	29±7.3*
AST (U/L)	37±5.0	37±5.8	35±8.1	38±3.3
GGT (U/L)	3.0±0.78	3.1±0.51	2.4±0.47	1.1±0.48**
Glucose (mg/dL)	103±5.5	104±3.0	105±4.8	103±6.0
Females				
Albumin (g/dL)	3.6±0.12	3.5±0.09	3.4±0.11	3.5±0.24
Total protein (g/dL)	5.4±0.12	5.6±0.19	5.5±0.27	5.4±0.43
Globulin (g/dL)	1.8±0.19	2.1±0.15	2.0±0.26	2.0±0.21
A/G ratio	2.05±0.28	1.73±0.12	1.71±0.24	1.78±0.14
Urea nitrogen (mg/dL)	14.2±1.8	13.3±0.9	13.5±3.7	13.3±2.0
Creatinine (mg/dL)	0.5±0.07	0.5±0.08	0.5±0.08	0.5±0.05
ALP (U/L)	94±17.2	98±10.9	83±11.3	58±10.9**
ALT (U/L)	39±5.6	43±7.3	40±3.6	33±5.1
AST (U/L)	33±6.6	42±5.0	33±2.8	43±11.4
GGT (U/L)	2.9±0.64	2.5±0.73	2.0±0.43	1.4±0.60**
Glucose (mg/dL)	105±5.0	105±1.5	103.±1.8	96±5.4**

N = 5/sex/dose except 150 mg/kg/day females where N = 4.
* = $p \leq 0.05$; ** = $p \leq 0.01$.
A/G ration = albumin to globulin ratio, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, GGT = gamma glutamyl transferase

There were no test substance-related effects on urinalyses.

Gross pathology, organ weights, and histopathology:

There were no treatment-related effects observed during gross necropsy evaluations. Liver weights relative to final body weights were higher ($p<0.01$) in the 150 mg/kg/day group males and females (Table 7). Minimal hepatocellular hypertrophy was noted in the liver of 150 mg/kg/day group animals. There were no other test substance-related histological changes.

Table 7: Liver weights and histopathology findings at terminal sacrifice (week 13) for DCSA (MON 52708) in a 90-day dog study (mean±SD)

	Males				Females			
	0 mg/kg/day	15 mg/kg/day	50 mg/kg/day	150 mg/kg/day	0 mg/kg/day	15 mg/kg/day	50 mg/kg/day	150 mg/kg/day
Absolute liver weight (g)	278.72± 49.23	306.35± 14.21	327.17± 37.32	332.11± 43.78	232.86± 35.62	238.20± 27.07	257.86± 42.85	254.11± 30.37
Relative (to body) liver weight (g)	2.487± 0.237	2.629± 0.204	2.781± 0.070	3.300± 0.208**	2.510± 0.376	2.535± 0.126	2.700± 0.170	3.155± 0.186**
Hepatocellular hypertrophy (incidence)	0	0	0	5	0	0	0	4

N = 5 for all dose groups except 150 mg/kg/day females, where N = 4.
** = $p \leq 0.01$.

IV. EVALUATION, SUMMARY AND CONCLUSIONS BY REGULATORY AUTHORITY

C. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U.S. EPA

D. REVIEWER'S COMMENTS:

RELIABILITY RATING: Reliable (Acceptable/Guideline)

This study is fully compliant with OECD.409

The test-substance results observed in this 90 day study of the toxic effects of MON 52708 administered orally (capsule) to Beagle dogs were similar between males and females and limited to the highest dose group (150 mg/kg/day), except for two instances in which small differences were observed in the 50 mg/kg/day group. These two effects observed at 50 mg/kg/day were 1) slightly increased abnormal excreta (soft faeces) and 2) a slight (approximately 10%) increase in APTT in males; both of these effects were judged to be non-adverse.

The study summary prepared by the registrant did not attribute the death of the female in the 150 mg/kg/day group to treatment. However, this review disagrees with the quoted statement from the registrant. The emesis in the sacrificed female, which was consistent with emesis seen in other treated dogs, resulted in electrolyte imbalances and dehydration which were the cause of the early sacrifice. The condition of this dog and the sacrifice are attributed to treatment.

Revised by U.S. Environmental Protection Agency

A Prenatal Developmental Toxicity Study of MON 52708 in Rats

Report: IIA 5.8/19. Coder, P. S. (2007). A Prenatal Developmental Toxicity Study of MON 52708 in Rats. WIL Research Laboratories, LLC, Ashland, OH, unpublished report WI-2007-001 / WIL-50309, MRID 47899519.

Dates of Work: January 11, 2007 – April 11, 2007

Guidelines: OECD 414
EPA OPPTS 870.3700
Deviations: None.
PMRA DACO 4.5.2

GLP: Yes Signed and dated GLP Compliance, Data Confidential Statements, Quality Assurance and Flagging Statement were provided

Executive Summary:

In a prenatal developmental toxicity study (MRID 47899519) groups of 25 bred female Crl:CD(SD) rats were administered MON 52708 (purity 97.9%; Lot/batch# GLP-0603-16958-T) by oral gavage at doses of 0, 10, 30 and 100 mg/kg/day from gestation days 6 through 19. The doses for this study were based on a previous prenatal developmental toxicity dose range-finding study (MRID47899518).

All animals were observed twice daily for mortality and moribundity, and individual clinical observations were recorded from gestation days 0 through 20. Animals were also observed for signs of toxicity approximately 1 hour following dose administration. Body weights and food consumption were recorded on gestation days 0 and 6-20. On gestation day 20, a laparohysterectomy was performed on each female. The uteri, placentae and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were weighed, sexed and examined for external, visceral and skeletal malformations and developmental variations.

All females survived to the scheduled necropsy on gestation day 20; there were no test article-related clinical or macroscopic findings at any dose level. Mean maternal body weights, body weight gains, net body weights, net body weight gains, gravid uterine weights and food consumption in all test article-treated groups were generally similar to those in the control group.

No test article-related effects on intrauterine growth, survival or fetal morphology were observed at any dose level.

Doses in this study were based upon toxicity in a pilot study (MRID47899518, see Appendix). In the pilot study clinical observations at 200 mg/kg/day included salivation, red and/or clear material around the mouth and/or nose, and yellow or brown material around the genital area. Fetal body weights were decreased 14% in the 200 mg/kg/day group compared to controls.

The maternal and developmental NOAELs are both 100 mg/kg/day, the highest dose tested. A LOAEL was not determined.

This study is classified **totally reliable (acceptable/guideline) when considered in conjunction with the rangefinding study** (MRID47899518) and satisfies the guideline requirements (EPA OPPTS guideline 870.370 and OCED guideline 414).

I. MATERIALS AND METHODS

A. Materials

1. Test material:	DCSA (MON 52708);
Description:	White powder
Lot/Batch#:	GLP-0603-16958-T
Purity:	97.9%
CAS #:	Not given
Compound Stability:	The test substance was considered stable at room temperature
Structure:	Not available

2. Test animals

Species:	Rats, females (virgin, sexually mature)	
Strain:	Crl:CD® (SD)	
Age/ weight at study initiation:	Approximately 12 weeks old at the initiation of dose administration. Females: 227 – 299 g	
Source:	Charles River Laboratories, Inc., Raleigh, NC	
Housing	Individually in clean, stainless steel, wire-mesh cages suspended above cage-board.	
Diet:	Certified Rodent LabDiet® 5002 (PMI Nutrition International, LLC), *ad libitum*. The feed batch was analyzed for contaminants. No unacceptable levels of contaminants were present.	
Water:	Reverse osmosis-purified (on site) drinking water, *ad libitum*	
Environmental conditions:	**Temperature:**	22°C ± 3°C
	Humidity:	50% ± 20%
	Air changes:	At least 10/hr
	Photoperiod:	12 hrs Dark /12hrs light
Acclimation period:	14 days	

B. Study Design and Methods:

The test material was suspended in corn oil and administered by gavage to groups of 25 sexually mature, virgin female Crl:CD(SD)® rats at dose levels of 10, 30, or 100 mg/kg/day from days 6 through 19 of pregnancy at a dose volume of 5 mL/kg. A concurrent control group of 25 female rats received the corn oil vehicle only on a comparable regimen. The rats were observed twice daily for signs of moribundity/mortality. Detailed clinical signs were recorded daily. Body weights and food consumption were recorded on day 0 and daily from day 6-20. On day 20 of pregnancy all surviving females were sacrificed and subjected to a laparohysterectomy. Uteri and ovaries were examined and the number of corpora lutea, the number and location of all foetuses, early and late resorptions, and the number of implantation sites were recorded. The placentas were also examined. The liver, kidneys, and spleen were weighed for each female at the scheduled necropsy. Each foetus was weighed and sexed and a detailed examination for external, visceral, and skeletal malformation and developmental variations was conducted to include an examination of the eyes, palate, and external orifices.

TABLE 1. Study Design

Group No.	Test Substance	Dose Level (mg/kg/day)[a]	Dose Volume (ml/kg)	Number of Females
1	Vehicle	0	5	25
2	MON 52708	10	5	25
3	MON 52708	30	5	25
4	MON 52708	100	5	25

a = A correction factor of 1.023 was used to account for test substance purity.

Mating: One hundred twenty-six sexually mature, virgin female Crl:CD(SD) rats received in good health from Charles River Laboratory were observed twice daily for mortality and general changes in appearance and behavior. At the conclusion of the acclimation period, all available females were weighed and examined in detail for physical abnormalities. Each animal judged to be in good health and meeting acceptable body weight requirements (a minimum of 220 g) was placed in a suspended wire-mesh cage with a resident male from the same strain and source for breeding. The selected females were approximately 12 weeks old when paired for breeding. Resident males were untreated, sexually mature rats utilized exclusively for breeding. Positive evidence of mating was confirmed by the presence of a vaginal copulatory plug or the presence of sperm in a vaginal lavage. Each mating pair was examined daily. The day on which evidence of mating was identified was termed gestation day 0 and the animals were separated.

Fetal examination: Each viable fetus was examined externally, individually sexed, weighed, tagged for identification, and euthanized by hypothermia followed by an intrathoracic injection of sodium pentobarbital (if necessary). Fetal tags contained the WIL study number, the female number and the fetus number. The detailed external

examination of each fetus included, but was not limited to, an examination of the eyes, palate and external orifices, and each finding was recorded.

Each viable fetus was subjected to a visceral examination using a modification of the Stuckhardt and Poppe fresh dissection technique to include the heart and major blood vessels (Stuckhardt and Poppe, 1984). The sex of each fetus was confirmed by internal examination. Heads from approximately one-half of the fetuses in each litter were placed in Bouin's fixative for subsequent soft-tissue examination by the Wilson sectioning technique (Wilson, 1965). The heads from the remaining one-half of the fetuses were examined by a mid-coronal slice. All carcasses were eviscerated and fixed in 100% ethyl alcohol.

Following fixation in alcohol, each fetus was stained with Alizarin Red S (Dawson, 1926) and Alcian Blue (Inouye, 1976). External, visceral and skeletal findings were recorded as developmental variations (alterations in anatomic structure that are considered to have no significant biological effect on animal health or body conformity and/or occur at high incidence, representing slight deviations from normal) or malformations (those structural anomalies that alter general body conformity, disrupt or interfere with normal body function, or may be incompatible with life).

The fetal developmental findings were summarized by: 1) presenting the incidence of a given finding both as the number of fetuses and the number of litters available for examination in the group; and 2) considering the litter as the basic unit for comparison and calculating the number of affected fetuses in a litter on a proportional basis as follows:

$$\text{Summation Per Group (\%)} = \frac{\sum \text{Viable Fetuses Affected/Litter (\%)}}{\text{No. Litters/Group}}$$

Where:

$$\text{Viable Fetuses Affected/Litter (\%)} = \frac{\text{No. Viable Fetuses Affected/Litter}}{\text{No. Viable Fetuses/Litter}} \times 100$$

II. RESULTS AND DISCUSSION

General observations:

All females in the control, 10, 30, and 100 mg/kg/day groups survived to the scheduled necropsy and there were no clinical signs of toxicity noted at the daily examinations or one hour following administration of the test material at any dose level.

Maternal body weight and food consumption:

Group mean maternal body weights were comparable between test groups and the control group. Mean body weight gain in the 100 mg/kg/day group was statistically significantly ($p<0.01$) lower than the control group during gestation day 12-13 only; this finding was transient and was therefore not considered related to test article administration. Food consumption was unaffected by test article administration.

Maternal necropsy data:

No test article-related adverse internal findings were observed in the study.

Uterine and foetal data:

Intrauterine growth and survival were unaffected by test article administration. As shown in Table 2, there were no differences between treated and control values for viable foetuses (% per litter), postimplantation loss (% per litter), corpora lutea/dam, mean foetal body weights and foetal sex distributions.

TABLE 2. Cesarean section observations [a]

Observation	Dose (mg/kg bw/day)			
	0	10	30	100
# Animals assigned (mated)	25	25	25	25
# Animals pregnant	25	25	25	24
# Nonpregnant	0	0	0	1
Maternal wastage				
No. died	0	0	0	0
No. died pregnant	0	0	0	0
No. died nonpregnant	0	0	0	0
No. aborted	0	0	0	0
No. Premature delivery	0	0	0	0
Total No. corpora lutea Corpora lutea/dam	433 17.3 ± 2.32	413 16.5 ± 2.43	412 16.5 ± 1.58	421 17.5 ± 1.69
Mean Corpora lutea/dam	17.3±2.3[b]	16.5±2.4	16.5±1.6	17.5±1.7
Total No. implantations (Implantations/dam)	401 16.0 ± 1.40	374 15.0 ± 3.62	392 15.7 ± 1.46	390 16.3 ± 1.70
Total No. litters	375	357	372	377
Total No. live fetuses (Live fetuses/dam)	375 15.0 ± 1.47	357 14.3 ± 3.76	372 14.9 ± 1.81	377 15.77 ± 2.27
Total No. dead fetuses	0	0	0	0
Viable foetuses (% per litter)	93.5±4.6	94.5±8.1	94.8±5.7	96.4±5.9
Total No. resorptions				
Early	26	17	20	13
Late	0	0	0	0
Mean combined fetal weight (g)	3.7±0.20	3.7±0.20	3.7±0.24	3.6±0.27
Males	3.7±0.21	3.8±0.25	3.8±0.23	3.7±0.26
Females	3.6±0.21	3.6±0.19	3.6±0.24	3.5±0.24
Foetal sex distribution (%M/%F)	53.2/46.8	52.0/48.0	49.4/50.6	51.3/48.7
Sex ratio (% male)	53±13.14	52.0±15.87	49.4±13.10	51.3±11.80
Preimplantation loss (%)	6.7±6.80	9.0±18.56	4.6±6.57	7.1±8.04
Postimplantation loss (%)	6.5±4.56	5.5±8.13	5.2±5.65	3.6±5.92

a Data obtained from pages 62-65 in the study report. b Mean±SD
None significantly different from control group

Foetal dysmorphology:

There were no biologically meaningful or statistically significant differences in the number of litters with malformations in any of the treatment groups when compared with controls. Only three malformations were observed in the study: an open eyelid and mandibular and maxillary agnathia in a control foetus; an open eyelid, shorter than normal body, and craniorhachischisis in a 10 mg/kg/day foetus, and one skeletal malformation in the 200 mg/kg/day group. The frequency of occurrence of malformations in this study was considered spontaneous in origin since the number of litters and foetuses showing malformations was not dose related and since the frequency of occurrence was so low. The skeletal developmental variations in the DCSA treated groups were similar in type and incidence to those noted in the control group. Thus, no foetal malformations or variations were attributed to the test article.

V. EVALUATION, SUMMARY AND CONCLUSIONS BY REGULATORY AUTHORITY

E. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U.S. EPA

F. REVIEWER'S COMMENTS:

RELIABILITY RATING: Reliable (Acceptable/Guideline).
This study is fully compliant with OECD 414.

No maternal or fetal toxicity occurred in this study. This study should be evaluated in conjunction with the rangefinding study (MRID47899518, see Appendix). In the pilot study clinical observations at 200 mg/kg/day included salivation, red and/or clear material around the mouth and/or nose, and yellow or brown material around the genital area. Fetal body weights were decreased 14% in the 200 mg/kg/day group compared to controls. No external malformations or variations were noted at 200 mg/kg/day. The dose of 500 mg/kg/day resulted in the deaths of 2/8 dams.

C. CONCLUSION

In the absence of maternal or developmental toxicity in this study, the NOAEL for DCSA (MON 52708) for maternal and developmental toxicity in this study was 100 mg/kg/day, the highest dose tested.

Deficiencies

No deficiencies were noted in this study.

APPENDIX

Citation: IIA 5.8.18. Coder, P.S. (2007). A Dose Range-Finding Prenatal Developmental Toxicity Study of MON 52708 in Rats. WIL Research Laboratories LLC, Ashland, OH, unpublished report WI-2006-051/WIL-50308. MRID 47899518.

Executive Summary for Rangefinding Study: In a prenatal development toxicity rangefinding test (MRID47899518) groups of 8 bred female Crl:CD(SD) rats were administered by MON 52708 (purity 97.9%; Lot/batch no GLP-0603-16958-T) by oral gavage at doses of 0, 50, 200, 500 or 1000 mg/kg/day from gestation days 6-19.

All animals were observed twice daily for mortality and moribundity, and individual detailed clinical observations were recorded from gestation days 0 through 20. Animals were also observed for signs of toxicity at the time of dose administration and approximately 1 hour following dose administration. Body weights and food consumption were recorded on gestation days 0 and 6-20. On gestation day 20, a laparohysterectomy was performed on each surviving female. The fetuses were weighed, sexed and examined for external malformations and developmental variations.

In the 1000 mg/kg/day group, 7 of the females were found dead and 1 female was euthanized in extremis on gestation day 7, 8 or 9. In the 500 mg/kg/day group, 2 females were found dead, 1 each on gestation days 8 and 10. All other females survived to the scheduled necropsy.

Clinical findings for surviving females in the 200 and 500 mg/kg/day groups included salivation, red and/or clear material around the mouth and/or nose, and yellow or brown material around the genital area. In addition, in the 500 mg/kg/day group, excessive pawing and wiping of the mouth on the cage were noted.

Mean maternal body weight losses and/or lower mean body weight gains and lower food consumption, mean gravid uterine weights, net body weights and/or net body weight gains (relative to the control group) were generally noted in the 200 and 500 mg/kg/day groups throughout the treatment period. Body weight gain for the 200 mg/kg/day group was 92 g vs 117 g in controls. Body weights were also reduced in the 500 mg/kg/day group, though this was in part due to the 100% resorptions at that dose.

At the scheduled necropsy, no remarkable macroscopic findings were noted in the surviving dams at any dose level. Mean absolute liver weights in the 200 and 500 mg/kg/day groups were 7.0% and 19.0% lower than the control group value, respectively. In addition, slightly higher mean absolute spleen and kidney weights (16.7% and 11.6%, respectively) were noted in the 500 mg/kg/day group when compared to the control group values.

Evaluation of laparohysterectomy parameters in the 1000 mg/kg/day group was precluded by the death of all females in this group. Surviving females in the 500

mg/kg/day group had early resorptions of all litters. In the 200 mg/kg/day group, mean fetal weight was decreased 14% compared to controls. No malformations or developmental variations were noted in any fetuses in the control or test article-treated groups following an external examination.

Revised by the U.S. Environmental Protection Agency

STUDY TYPE: A dose range-finding prenatal developmental toxicity study of MON 52724 in rats

REPORT: IIA 5.8/17. Coder, P. S. (2009d). An oral (gavage) dose range-finding prenatal developmental toxicity study of MON 52724 in rats. WIL Research Laboratories LLC, unpublished report WI-09-096/WIL-50368

SPONSOR: Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, MO 63167

Guidelines: None – rangefinding study.
Deviations: None.
PMRA DACO 4.5.2

GLP: Yes

EXECUTIVE SUMMARY:

In a dose range-finding toxicity study (MRID 47899520) four groups of eight bred female Crl:CD(SD) rats per dose group were exposed to DCGA (MON 52724 Purity 96.3%; Lot/batch No GLP-0903-19699-T) by gavage with corn oil at doses of 0, 50, 200, 500, and 1000 mg/kg/day. Animals were observed twice daily for moribundity and mortality and individual detailed clinical observations were recorded from day 0 through gestation day 20. Body weights and food consumption were recorded from gestation days 0 and 6-20. On gestation day 20, a laprohysterectomy was performed on each of the surviving animals and the uteri, placentae, ovaries were examined, and the number of fetuses, early and late resorptions, total implantations and corpora lutea were recorded. Fetuses received an external examination but not a soft tissue or skeletal examination.

Mean body weights were 4.0% to 6.6% lower during gestation days 13-20 in the 500 mg/kg/day group and 4.4% to 12.1% lower during gestation days 12-20 in the 1000 mg/kg/day group. Five of the eight females in the 1000 mg/kg/day group died or were euthanized *in extremis* during gestation days 12-19. Clinical findings in dams included rales and red or clear material on body surfaces at doses of 200 mg/kg/day and above. There were no effects observed on uterine growth, survival, external malformations or variations.

Because this rangefinding study was not intended to fulfill a guideline requirement, NOAELs and LOAELs are not assigned. This study is suitable for use in dose selection for a definitive guideline study.

I. MATERIALS AND METHODS

MATERIALS

1. Test material: MON 52724

Description: Off-white fine powder

Lot/Batch#: GLP-0903-19699-T

Purity: 96.3%

CAS #: Not provided

Stability of test compound: Validated stability date: March 17, 2009

2. Vehicle and/or positive control: Vehicle: Corn oil (lot no. 058K0070, exp. dates: 11 February and 20 March 2010, manufactured by Sigma Aldrich, St. Louis, MO).

3. Test system:

Species: Virgin female rats

Strain: Crj:CD(SD)IGS [SPF]

Age: 70 days upon receipt

Weight at dosing: 255-261g

Source: Charles River Laboratories, Inc., Raleigh, NC

Acclimation period: 14 days

Diet: Certified Rodent LabDiet® 5002, PMI Nutrition International, LLC *ad libitum*

Water: Tap water *ad libitum*

Housing: Singly in stainless steel mesh cages

Properly maintained? Yes

4. Environmental conditions:

Temperature: 22±3°C

Humidity: 50%±20%

Air changes: 10/hour

Photoperiod: 12 hours light / 12 hours darkness

5. Test compound administration

Table 1 – Group assignments and dosage

Group Number	Test Substance	Dose Level (mg/kg/day)	Test substance Concentration (mg/mL)
1 (control)	Vehicle	0	N/A
2	MON 52724	50	10
3	MON 52724	200	40
4	MON 52724	500	100
5	MON 52724	1000	200

Source: page 18 of the Report

6. In life dates: March 17, 2009 – April 13, 2009

Methods

7. Treatment: The vehicle and test substance formulations were administered orally by gavage, via an appropriately sized flexible, Teflon-shafted, stainless steel ball-tipped dosing cannula, once daily during gestation days 6-19. The dose volume for all groups was 5 mL/kg. Individual doses were based on the most recently recorded body weights

to provide the correct mg/kg/day dose. All animals were dosed at approximately the same time each day. Table 1 above displays the group sizes and dosage levels.

Mating: The rats were paired for mating in the home cage of the male. Following positive evidence of mating, the females were returned to individual suspended wire-mesh cages; nesting material was not required as the females were euthanized prior to the date of expected parturition.

Fetal evaluations: Fetal examinations were performed blind to treatment group. A detailed external examination of each fetus included an examination of the eyes, palate and external orifices. Skeletal and soft tissue examinations were not conducted. Findings were recorded as either developmental variations (alterations in anatomic structure that are considered to have no significant biological effect on animal health or body conformity and/or occur at high incidence, representing slight deviations from normal) or malformations (those structural anomalies that alter general body conformity, disrupt or interfere with normal body function, or may be incompatible with life). Each fetus was weighed, sexed, euthanized by hypothermia followed by an intrathoracic injection of sodium pentobarbital (if necessary), and discarded.

II. Results:

Maternal clinical signs:

Five of the eight females in the 1000 mg/kg/day group died or were euthanized *in extremis* during gestation days 12-19. Surviving females had rales approximately 1 hour following treatment in the 200 (3/8 females), 500 (7/8), and 1000 mg/kg/day groups (3/8). The majority of females in the 200 mg/kg/day and higher groups had dose-related incidences of red and clear material on various body surfaces.

Maternal body weight and food consumption:

Reductions in mean maternal body weight gains and food consumption were generally noted throughout the treatment period in the 500 and 1000 mg/kg/day groups (Table 2). Mean net body weights (gestation day 20 dam body weights exclusive of the weight of the uterus and contents) were decreased (but not statistically significantly) at gestation day 20 for these same groups compared to controls.

Table 2 – Summary of maternal body weight during gestation						
Group		0 mg/kg/day	50 mg/kg/day	200 mg/kg/day	500 mg/kg/day	1000 mg/kg/day
Day 0	MEAN	257	258.	255	258.	263
	S.D.	20.4	13.2	12.9	10.9	16.9
Day 6	MEAN	290.	288.	291.	295.	294
	S.D.	22.0	18.8	13.5	14.8	23.2
Day 7	MEAN	291.	292.	292.	294.	296
	S.D.	21.4	19.8	14.4	13.6	21.2
Day 8	MEAN	297.	294.	297.	297.	300
	S.D.	20.9	20.4	15.6	15.1	21.6
Day 9	MEAN	299.	297.	301.	299.	301
	S.D.	19.8	22.8	15.4	14.4	20.8
Day 10	MEAN	307	303	308	303	306
	S.D.	21.1	22.2	15.0	15.1	21.2
Day 11	MEAN	313	312	314	306	308
	S.D.	24.1	25.0	19.1	15.5	19.7
Day 12	MEAN	316	313	316	307	302
	S.D.	21.8	25.1	21.6	19.9	29.9
Day 13	MEAN	322	319	318	309	283*
	S.D.	22.8	34.4	21.1	22.7	27.2
Day 14	MEAN	326	326	321	312	287*
	S.D.	24.2	23.4	20.3	22.4	37.3
Day 15	MEAN	335	335	330	318	319
	S.D.	25.6	25.8	20.6	22.1	20.1
Day 16	MEAN	346	346	341	329	346
	S.D.	26.5	24.8	21.7	21.6	17.7
Day 17	MEAN	361	363	357	342	340
	S.D.	28.1	26.6	21.3	20.4	18.8
Day 18	MEAN	375	380	372	357	356
	S.D.	30.7	28.9	24.9	22.4	23.6
Day 19	MEAN	390	397	388	367	363
	S.D.	34.3	28.0	24.2	25.1	31.2
Day 20	MEAN	407	418	405	380	389
	S.D.	42.0	31.1	23.4	26.7	22.7

Source: Pages 49-52 of Report
* = Significantly different from the control group at 0.05 using Dunnett's test

Maternal necropsy data and organ weights:

No test substance-related macroscopic findings were noted for females at any dose level at the scheduled necropsy on gestation day 20 (Table 3). No changes in mean organ weights were considered test substance related.

Table 3 – Maternal Organ weight

Organ	GROUP	0 MG/KG/DAY	50 MG/KG/DAY	200 MG/KG/DAY	500 MG/KG/DAY	1000 MG/KG/DAY
LIVER	MEAN	18.08	17.32	16.52	14.97*	16.70
	S.D.	1.018	1.976	1.320	2.054	0.044
KIDNEYS	MEAN	2.35	2.30	2.24	2.22	2.23
	S.D.	0.120	0.322	0.139	0.207	0.365
SPLEEN	MEAN	0.84	0.69	0.68	0.69	0.77
	S.D.	0.186	0.107	0.119	0.128	0.120

Source: Page 67 of Report
* = Significantly different from the control group at 0.01 using Dunnett's test

Uterine and foetal data

Fetal growth and survival were unaffected by maternal test substance administration at all dose levels (Table 4 and 5). No malformations or variations were attributed to treatment at any dose.

Table 4 – Fetal Data

Group		Sex		Viable Fetuses	Dead Fetuses	Resorptions		Post Implantation Loss	Implantation Sites	Corpora Lutea	Pre-implantation Loss	Fetal weight (g)	No. of Gravid females
		M	F			Early	Late						
1	TOTAL	54	61	115	0	6	0	6	121	131	10	NA	8
	MEAN	6.8	7.6	14.4	0	0.8	0	0.8	15.1	16.4	1.3	3.6	
	S.D.	3.2	2.97	3.93	0	0.46	0	0.46	3.83	1.92	2.43	0.19	
2	TOTAL	51	58	109	0	4	0	4	113	115	2	NA	7
	MEAN	7.3	8.3	15.6	0	0.6	0	0.6	16.1	16.4	0.3	3.8	
	S.D.	2.43	2.56	1.99	0	0.53	0	0.53	1.68	2.3	0.76	0.19	
3	TOTAL	61	67	128	0	5	0	5	133	126	8	NA	8
	MEAN	7.6	8.4	16	0	0.6	0	0.6	16.6	18	1.1	3.7	
	S.D.	1.41	2.67	2.14	0	0.92	0	0.92	1.69	0.58	1.46	0.18	
4	TOTAL	61	61	122	0	4	0	4	126	142	16	NA	8
	MEAN	7.6	7.6	15.3	0	0.5	0	0.5	15.8	17.8	2	3.6	
	S.D.	2.07	2.13	1.67	0	0.53	0	0.53	1.83	2.19	2.27	0.35	
5	TOTAL	30	19	49	0	1	0	1	50	52	2	NA	3
	MEAN	10	6.3	16.3	0	0.3	0	0.3	16.7	17.3	0.7	3.5	
	S.D.	1	2.08	1.15	0	0.58	0	0.58	1.53	1.53	0.58	0.26	

Source: Page 68 of Report

Table 5 - Summary of foetal observations at study termination for a developmental toxicity study with rats receiving DCGA					
	Dose (mg/kg/day)				
	0	50	200	500	1000
Viable foetuses (% per litter)[a]	14.4±3.9	15.6±2.0	16.0±2.1	15.3±1.7	16.3±1.2
Postimplantation loss (% per litter)[a]	0.8±0.5	0.6±0.5	0.6±0.9	0.5±0.5	0.3±0.6
Corpora lutea/litter[a]	16.4±1.9	16.4±2.3	18.0±0.6	17.8±2.2	17.3±1.5
Foetal body weights (g)[a]	3.6±0.2	3.8±0.2	3.7±0.2	3.6±0.4	3.5±0.3
Foetal sex distribution (%M/%F)	46.6/53.4	46.8/53.2	48.6/51.4	50.1/49.9	61.7/38.3

Source: Page 69-72 of Report
[a] Mean±SD.
No values were statistically significantly different from controls.

III. EVALUATION, SUMMARY, and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticices Program/U.S. EPA

B. CONCLUSIONS: Test substance-related toxicity was evidenced by reductions in mean maternal body weights, body weight gains, net body weights, net body weight gains, and food consumption at dose levels of 500 and 1000 mg/kg/day when MON 52724 was administered orally by gavage to pregnant Crl:CD(SD) rats. In addition, the maximum tolerated dose was exceeded at 1000 mg/kg/day based on maternal mortality and moribundity. Clinical findings in dams included rales and red or clear material on body surfaces at doses of 200 mg/kg/day and above. Intrauterine growth and survival and fetal morphology were unaffected by maternal test substance administration at all dose levels following an external examination.

Because this rangefinding study was not intended to fulfill a guideline requirement, NOAELs and LOAELs are not assigned. This study is suitable for use in dose selection for a definitive guideline study.

No deficencies were noted in this rangefinding study.

Revised by U.S. Environmental Protection Agency

A developmental toxicity study of MON 52708 in rabbits

Report: IIA 5.8/21. Coder, P.S. (2009b). A prenatal developmental toxicity study of MON 52708 in rabbits. WIL Research Laboratories, LLC, unpublished report WI-2007-031/WIL-50330

Dates of work: June 2, 2009 – July 1, 2009

Guidelines: U.S. EPA OPPTS 870.3700, OECD 414
Deviations: None.
PMRA DACO 4.5.3

GLP: Yes

Executive Summary:

In a developmental toxicity study (MRID 47899522), groups of twenty-five mated female New Zealand white rabbits were exposed to DCSA (MON 52708) (Purity 97.7%; Lot/batch No GLP-0603-16958-T) by gavage from gestation days 6-28 at doses of 0, 10, 25, or 65 mg/kg/day. All animals were observed twice daily for moribundity and mortality and individual detailed clinical observations, body weights, and food consumption were recorded. On gestation day 29, a laprohysterectomy was performed on each surviving females and the uteri, placentae, ovaries were examined, and the number of fetuses, early and late resorptions, total implantations and corpora lutea were recorded.

One control female and one female in the 65 mg/kg/day group died with cause of death undetermined. One female in the 10 mg/kg/day group aborted. All treatment groups had decreased defecation. There were no toxicologically significant test substance related effects observed on survival, clinical signs, body weight, food consumption, intrauterine growth, pup survival, external malformations or mophology of fetuses.

Although no toxicity occurred in this study at the high dose of 65 mg/kg/day, the does could not have tolerated a much higher dose because 100 mg/kg/day was found to be a maternally lethal dose in the rangefinding study (MRID 47899521, see Appendix). Therefore, this study is classified **totally reliable (acceptable/guideline) when considered in conjunction with the rangefinding study** and satisfies the guideline requirements for a developmental toxicity study in rabbits (OPPTS 870.3700, OECD 414).

The maternal and developmental NOAELs are 65 mg/kg/day, the highest dose tested. The maternal and developmental LOAELs were not determined.

MATERIALS AND METHODS:

Test material:

Description:	White Powder
Lot/batch #:	GLP-0603-16958-T
Purity:	97.7% a.i.
Compound stability:	Stable at room temperature for a year
CAS #of TGAI:	None provided
Structure:	None available

Test animals:

Species:	Rabbit	
Strain:	New Zealand White	
Age/weight at study initiation:	Approximately 6 months. 2968-4057 g at gestation day 0	
Source:	Covance Research Products, Inc., Kalamazoo, MI	
Housing:	Housed individually, in steel cages suspended above bedding	
Diet:	PMI Nutrition International LLC, Certified Rabbit LabDiet 5322	
Water:	Reverse osmosis treated municipal water	
Environmental conditions:	**Temperature:**	66±5°F
	Humidity:	50%±20%
	Air changes:	10/hr
	Photoperiod:	12 hr cycle (0600 to 1800 exposure to light)
Acclimation period:	Not discussed	

Mating: No details regarding the mating of the rabbits were provided. All rabbits used in the study were impregnated prior to arrival at the laboratory.

Fetal evaluations: The fetuses were weighed, sexed, and examined for external, visceral and skeletal malformations and developmental variations. The fetuses were examined in the following manner:

Fetal examinations were performed blind to treatment group. Each viable fetus was examined externally, individually weighed, euthanized by hypothermia followed by an intrathoracic injection of sodium pentobarbital (if necessary) and tagged for identification. Fetal tags contained the WIL study number, the female number, and the fetus number. The detailed external examination of each fetus included, but was not limited to, an examination of the eyes, palate and external orifices, and each finding was recorded. Crown-rump measurements, degrees of autolysis and gross examinations, if possible, were recorded for late resorptions and the tissues were discarded.

Each viable fetus was subjected to a visceral examination using a modification of the Stuckhardt and Poppe fresh dissection technique to include the heart and major blood vessels. The sex of each fetus was determined by internal examination. Fetal kidneys were examined and graded for renal papillae development. Heads from all fetuses were

examined by a mid-coronal slice. All carcasses were eviscerated and fixed in 100% ethyl alcohol.

Following fixation in alcohol, each fetus was macerated in potassium hydroxide and stained with Alizarin Red S. External, visceral and skeletal findings were recorded as developmental variations (alterations in anatomic structure that are considered to have no significant biological effect on animal health or body conformity and/or occur at high incidence, representing slight deviations from normal) or malformations (those structural anomalies that alter general body conformity, disrupt or interfere with normal body function, or may be incompatible with life).

The fetal developmental findings were summarized by:

1) presenting the incidence of a given finding both as the number of fetuses and the number of litters available for examination in the group; and
2) considering the litter as the basic unit for comparison and calculating the number of affected fetuses in a litter on a proportional basis as follows:

Summation per Group (%) = Viable Fetuses Affected/Litter (%) / No. Litters/Group

Where:

Viable Fetuses Affected/Litter (%) = Viable Fetuses Affected/Litter / Viable Fetuses/Litter x 100

II. Results:

General observations:

One female in the 65 mg/kg/day group was euthanized in extremis on gestation day 28 due to decreased food consumption and substantial body weight loss. No significant macroscopic findings were noted for this female at necropsy. One female in the control group was found dead on gestation day 27. All other animals survived to the scheduled necropsy. One 10 mg/kg/day group female aborted on gestation day 27. At necropsy, dark red areas on the lungs, two dead foetuses, and three late resorptions were observed for this female. Additionally, decreased body weight gains and reduced food consumption were noted for this animal beginning on gestation day 21.

Decreased defecation was noted in the 10, 25, and 65 mg/kg/day groups at a frequency higher that the control group. This did not always seem to correlate with decreased food consumption, but is not considered toxicologically significant.

Maternal body weight and food consumption:

Food consumption and body weight gain were decreased in the 65 mg/kg/day group compared to controls during gestation days 6-9. After this, body weight gain was similar to, or only slightly decreased compared to controls for the rest of the study. Overall, maternal body weights were unaffected by treatment (Table 2).

TABLE 2. Mean (±SD) maternal body weight gain (g) [a]				
Interval	**Control**	**LDT (10 mg/kg bw/day)**	**MDT (25 mg/kg bw/day)**	**HDT (65 mg/kg bw/day)**
Pretreatment: days 0-4	-9±73.2	-41±110.9	-40±82.1	-28±59.8
Treatment: days 13-14	36±25.6	24±33.0	26±34.8	6*±26.5
Posttreatment: days 28-29	23±29.5	6±35.6	-1±39.9	2±49.1
Corrected BW gain	-184.81±125.28	-158.7±157.64	229.3±186.93	-269.6±198.99

[a] Data obtained from pages *(insert pages 54-61)* in the study report.
* Statistically different ($p < 0.01$) from the control.

Uterine and foetal data:

Intrauterine growth and survival were unaffected by test substance administration at all dose levels tested (Table 3).

Table 3: Summary of foetal observations at study termination for developmental toxicity study with rabbits receiving DCSA				
	Dose (mg/kg/day)			
	0	**10**	**25**	**65**
Viable foetuses (% per doe)[a]	95.1±8.8	97.1±5.9	97.9±4.9	96.3±7.4
Postimplantation loss (% per doe)[a]	4.9±8.8	2.9±5.9	2.1±4.9	3.7±7.4
Corpora lutea/doe[a]	9.7±1.8	9.2±1.6	10.5±1.3	10.0±1.6
Foetal body weights (g)[a]	38.1±5.1	41.5±4.8	39.6±4.2	39.2±5.6
Foetal sex distribution (%M/%F)	47.6/52.4	48.1/51.9	52.1/47.9	50.2/49.8

[a] Mean±SD.
No values were statistically significantly different from controls.
Source: Table 5.8-46 of the Summary.

TABLE 4 Cesarean section observations [a] *[Include ±SD with mean values, as appropriate.]*				
Observation	**Dose (mg/kg bw/day)**			
	0	**LDT**	**MDT**	**HDT**
# Animals assigned (mated)	25	25	25	25
# Animals pregnant	22	22	24	21
# Nonpregnant	3	3	1	0
Maternal wastage				
No. died	--	--	--	--
No. died pregnant	1	--	--	1
No. died nonpregnant	--	--	--	--
No. aborted	--	1	--	--
No. Premature delivery	--	--	--	--
Total No. corpora lutea **Corpora lutea/dam**	214 9.7	202 9.2	253 10.5	209 10.0
Total No. implantations **(Implantations/dam)**	211 9.6	186 8.5	236 9.8	199 9.5
Total No. litters	22	22	24	21
Total No. live fetuses **(Live fetuses/dam)**	199 9.0	180 8.2	231 9.6	192 9.1
Total No. dead fetuses **(Dead fetuses/dam)**	0 0	0 0	0 0	0 0
Total No. resorptions	12	6	5	7
Early	8	4	5	5
Late	4	2	0	2
Litters with total resorptions	0	0	0	0
Mean fetal weight (g)	38.1	41.5	39.6	39.2
Males	38.5	41.7	39.8	39.8
Females	37.9	1.0	39.3	38.8
Sex ratio (% male)	47.6	48.1	52.1	50.2
Preimplantation loss (%)	1.1	8.2	6.4	4.2
Postimplantation loss (%)	4.9	2.9	2.1	3.7

[a] Source: Data obtained from pages 77-80 in the study report.

Foetal dysmorphology:

There were no malformations or variations which were attributed to treatment.

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:

Although no toxicity occurred in this study at the high dose of 65 mg/kg/day, the dose of 100 mg/kg/day was found to be a maternally lethal dose in the rangefinding study (MRID 47899521, see Appendix). Therefore, the does in this study could not have tolerated a

much higher dose, and this study is classified totallyreliable (acceptable/guideline) when considered in conjunction with the rangefinding study and satisfies the guideline requirements for a developmental toxicity study in rabbits (OPPTS 870.3700, OECD 414).

Reliability Rating: Reliable
This study is compliant with OECD 449(1997)

IV. REFERENCES

Dawson, A.B. A note on the staining of the skeleton of cleared specimens with Alizarin Red S. *Stain Technology* **1926**, *1*, 123-124.

Dunn, O.J. Multiple comparisons using rank sums. *Technometrics* **1964**, *6(3)*, 241-252.

Dunnett, C.W. New tables for multiple comparisons with a control. *Biometrics* **1964**, *20*, 482-491.

Kruskal, W.H.; Wallis, W.A. Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association* **1952**, *47*, 583-621.

National Research Council. *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, Commission on Life Sciences; National Academy Press: Washington, DC, **1996**.

Salewski, E. Färbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte. [Staining method for a macroscopic test for implantation sites in the uterus of the rat]. *Naunyn - Schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie* **1964**, *247*, 367.

Sawhney Coder, P. A Dose Range-Finding Prenatal Developmental Toxicity Study of MON 52708 in Rabbits (Study No. WIL-50329). WIL Research Laboratories, LLC, Ashland, OH, **2009.**

Snedecor, G.W.; Cochran, W.G. One Way Classifications; Analysis of Variance. In *Statistical Methods*, 7th ed.; The Iowa State University Press: Ames, IA, **1980**; pp 215-237.

Stuckhardt, J.L.; Poppe, S.M. Fresh visceral examination of rat and rabbit fetuses used in teratogenicity testing. *Teratogenesis, Carcinogenesis and Mutagenesis* **1984**, *4*, 181-188.

Woo, D.C.; Hoar, R.M. Apparent hydronephrosis as a normal aspect of renal development in late gestation of rats: the effect of methyl salicylate. *Teratology* **1972**, *6*, 191-196.

APPENDIX

Citation: IIA 5.8/20. Coder, P. S. (2009a). A dose range-finding prenatal developmental toxicity study of DCSA in rabbits. WIL Research Laboratories LLC, unpublished report WI-2007-005/WIL-50329. Sponsor: Monsanto Company, St. Louis, MO. MRID 47899521

Executive Summary for rangefinding study: In a dose range-finding toxicity test (MRID 47899521) groups of six mated female New Zealand white rabbits were exposed to DCSA (MON 52708) (Purity 97.7%; Lot/batch No GLP-0603-16958-T) administered orally by gavage with vehicle (0.5% carboxymethylcellulose) at doses of 0, 10, 30, 100, or 300 mg/kg/day. All animals were observed twice daily for moribundity and mortality and individual detailed clinical observations were recorded from day of the receipt through gestation day 29. Body weights were recorded pretreatment and from gestation days 4 and 6-29, and food consumption was recorded from gestation days 4 through 29. On gestation day 29, a laprohysterectomy was performed and the uteri, placentae, ovaries were examined, and the number of fetuses, early and late resorptions, total implantations and corpora lutea were recorded. Each fetus received an external examination.

All six females in the 300 mg/kg/day group were euthanized in extremis on gestation day 12 and 2 of 6 females were euthanized in extremis in the 100 mg/kg/day group, one each on gestation days 19 and 20. The euthanized rabbits all had decreased body weight; body weights were similar to controls for other rabbits. Developmental parameters were unaffected by treatment and no teratogenic effects were noted following an external fetal examination.

Revised by the U. S. Environmental Protection Agency

Study type: Two-Generation Reproductive Toxicity Study

Report: IIA 5.8/16. Coder, P. S. (2009). A Dietary Two-Generation Reproductive Toxicity Study of MON 52708 in Rats. WIL Research Laboratories, LLC, Ashland, OH, unpublished report WI-2007-017 / WIL-50326, MRID 47899517.

Dates of Work: March 22, 2007 – March 2, 2009

Guidelines: OECD 416
EPA OCSPP 870.3800
PMRA DACO 4.5.1

GLP: Yes Signed and dated GLP Compliance, Data Confidential Statements, Quality Assurance and Flagging Statement were provided

Executive Summary:

In a dietary two-generation reproductive toxicity study (MRID 47899517) DCSA (MON 52708) (purity 97.7%, Lot/Batch no., GLP-0603-16958-T) was administered continuously in the diet to groups of male and female Crl:CD(SD) rats (30/sex/group) at dose levels of 0, 50, 500 and 5000 ppm. One litter per dam was produced in each generation.

Mean test substance consumption for the F0 males was 4, 37 and 362 mg/kg/day and for F0 females was 4, 43 and 414 mg/kg/day during the premating period, 3, 34 and 323 mg/kg/day during gestation and 8, 78 and 610 mg/kg/day during lactation, for the 50, 500, and 5000 mg/kg/day groups, respectively.

Because all surviving offspring of the F0 animals in the 5000 ppm group were euthanized on PND 21 due to pup mortality and a high incidence of total litter loss among the dams, no offspring of the F0 animals in the 5000 ppm group were selected for the F1 generation. Mean test substance consumption for the F1 males was 4 and 41 mg/kg/day and for F1 females was 5 and 52 mg/kg/day during the premating period, 3 and 34 mg/kg/day during gestation and 8 and 79 mg/kg/day during lactation, for the 50 and 500 mg/kg/day groups, respectively.

Three additional groups of female rats (10/group) were included in this study for evaluation of clinical and histological pathology parameters. These non-mated satellite animals were administered either basal diet or the test substance in the diet for at least 90 consecutive days; dietary concentrations were 0, 50 and 500 ppm. No differences in clinical pathology or histological parameters were observed when comparing control and test substance-treated animal data. Mean test substance consumption for the satellite phase females in the 50 and 500 ppm groups was 4 and 42 mg/kg/day, respectively.

F0 and F1 parental survival was unaffected by test diet administration at all exposure levels. No remarkable clinical findings were noted at any exposure level tested in the F0 or F1 generations. Parental body weight and food consumption parameters were not adversely affected at exposure levels of 50 and 500 ppm in either generation. At an exposure level of 5000 ppm (evaluated only in the F0 generation), test substance-related reductions in mean body weight gain, food consumption and food efficiency were noted during the first month of test diet exposure, which resulted in lower mean body weights throughout the pre-mating period (females) or entire generation (males). Lower mean food consumption was also noted for the 5000 ppm group females throughout gestation and lactation.

There were no indications of adverse effects on reproductive performance in either the F0 or F1 generations. Male and female mating and fertility indices, male copulation indices, female conception indices, pre-coital intervals, spermatogenic endpoints, lengths of the estrous cycle and gestation, and live litter size were similar in all exposure groups. No test substance-related effects in gross pathology, organ weights or histopathology were noted in F0 or F1 parental animals. Additionally, ovarian follicle counts for the test substance-exposed F0 (5000 ppm, high-dose group) and F1 (500 ppm, high-dose group) females were similar to the control group values.

Test substance-related effects on pre-weaning offspring were noted at an exposure level of 5000 ppm (F1 pups) and included decreased pup survival during PND 0-1, 1-4 (pre-selection), 7-14 and 14-21 (due primarily to 7 females with total litter loss), clinical signs of toxicity (pale body, blackened ventral abdominal area, distended abdomen, uneven hair growth and desquamation) and lower body weights and weight gains during PND 1-21.

As a result of pup mortality and a high incidence of total litter loss among the F0 dams at 5000 ppm, all surviving offspring of the F0 animals in the 5000 ppm group were euthanized on PND 21; therefore, a dosage level of 5000 ppm group was not evaluated in the F1 generation.

At 500 ppm, mean F_1 male and female pup body weights on postnatal days 14 and 21 were reduced approximately -6% to -9% of controls; female pup body weight was also reduced at week 18 (-7%). Hyperkeratosis was noted upon histological evaluation of the F1 pups in the 5000 ppm group that had gross skin lesions or clinical findings of desquamation or uneven hair loss/hair growth.

No test substance-related effects on offspring survival, general physical condition, body weights, macroscopic pathology and organ weights were noted at exposure levels of 50 ppm for F1 or F2 pups. Mean ages and body weights on the day of attainment of balanopreputial separation and vaginal patency were unaffected by treatment in any group.

The parental NOAEL is 37 mg/kg/day and the parental LOAEL is 362 mg/kg/day based upon decreased body weight.

No reproductive toxicity was noted and the reproductive NOAEL is 362 mg/kg/day; the reproductive LOAEL was not attained.

The offspring NOAEL is 4 mg/kg/day and the offspring LOAEL is 37 mg/kg/day based upon decreased pup body weight in F_1 pups on postnatal days 14 and 21 (both sexes) and at week 18 (females only).

This study is classified totally reliable (acceptable/guideline) and satisfies the guideline requirement for a reproduction study (OECD 416, OPPTS 870.3800, PMRA DACO 4.5.1).

I. MATERIALS AND METHODS

A. Materials

1. **Test material:** DCSA (MON 52708);
   Description: White powder
   Lot/Batch#: GLP-0603-16958-T
   Purity: 97.7%
   CAS #: Not given
   Compound Stability: The test substance was considered stable at room temperature
   Structure: Not available

2. **Test animals**

Species:	Rats, sexually mature males and virgin females
Strain:	Sprague-Dawley rats: Crl:CD® (SD)
Age/ weight at study initiation:	(F0) Approximately 16 weeks old at the study initiation (F1) Approximately 13-15 weeks old at the study initiation (F0) Males: 382 – 632 g; Females: 219 – 357 g (F1) Males: 366 – 617 g; Females: 225 – 378 g
Source:	Charles River Laboratories, Inc., Raleigh, NC
Housing	Individually in clean, stainless steel, wired-mesh cages suspended above cage-board.
Diet:	Certified Rodent LabDiet® 5002 (PMI Nutrition International, LLC), *ad libitum*. The feed batch was analyzed for contaminants. No unacceptable levels of contaminants were present.

Water:		Reverse osmosis-purified (on site) drinking water, *ad libitum*
Environmental conditions:	**Temperature:**	22°C ± 3°C
	Humidity:	50% ± 20%
	Air changes:	At least 10/hr
	Photoperiod:	12 hrs Dark /12hrs light
Acclimation period:		15 days

3. **Dose selection rational:**

Dosage levels of the test substance were selected based on the preliminary results of a 90-day toxicity study (Kirkpatrick, 2009; WIL-50306), preliminary pharmacokinetic data from the Sponsor (Shah et al., Draft) and a dose range-finding prenatal developmental toxicity study (Sawhney, 2007).

4. **Dose preparation and analysis**:

Formulations were prepared weekly by mixing appropriate amounts of test substance with Rodent LabDiet #5002 (meal) and were stored at room temperature. Diet concentrations were adjusted for purity using a correction factor of 1.023. Separate batches of test diet were prepared for males and females at each exposure level. Prior to the start of the study, stability of the test substance was evaluated for a period of 8 and 14 days at room temperature storage and 16 days of frozen storage. Homogeneity (top, middle, and bottom) was evaluated in the 0 (control), 50 and 6000 ppm dosing formulations. Samples for concentration analysis were collected weekly from each dosing formulation including the control throughout the study and stored frozen. These samples were analyzed for test substance concentration during the first 3 weeks of the study and once per month for the reminder of the in-life phase.

Results

Homogeneity analysis: Results met the WIL SOP requirements for homogeneity, i.e., the RSD was 10% or less for the overall mean concentration with the concentration within 85% to 115% of target concentration.

Stability analysis: The dietary formulations were stable following 8 and 14 days of room temperature storage at concentrations of 50 and 6000 ppm, and following 14 days of room temperature storage and 16 days of frozen storage at a concentration of 25 ppm.

Concentration analysis: The analyzed formulations used for test substance administration met the WIL SOP requirement for concentration acceptability for diet admix formulations, i.e., the analyzed concentrations were within 15% of the target concentrations. No test substance was detected in the analyzed basal diet administered to the control group.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

B. Study Design and Methods:

The test material was administered in the diet to groups of Sprague-Dawley rats (Crl:CD(SD)), 30/sex/dose, at dietary levels of 0, 50, 500, or 5000 ppm for at least 70 days prior to animal breeding. Dosing for the F_0 females continued during mating, gestation, lactation, and until they were sacrificed after all litters were weaned. F_0 males received the basal or test diet until sacrificed following weaning of F_1 pups. Vaginal lavage was performed and the slides were evaluated microscopically to determine the stage of oestrous of each adult female for 21 days prior to pairing (on a 1:1 basis) and continuing until evidence of mating or until the end of the mating period. Body weights and food consumption were recorded weekly.

Detailed physical examinations were recorded weekly for all parental animals throughout the study period. All animals were observed twice daily for appearance, behavior, mortality, moribundity and clinical signs. F_1 litter sizes were standardized to eight pups (four/sex, where possible) on post natal day 4 (PND4).

Due to excessive toxicity and/or mortality during lactation, all surviving F_1 pups at 5000 ppm were euthanized at weaning. All other surviving F_1 offspring were weaned on post natal day 21 (PND 21) and 30/sex/group were selected to continue on basal or DCSA treatment through PND 70. These F_1 animals were bred to produce the F_2 litters.

Complete necropsies were performed on all F_0 and F_1 parental animals found dead, euthanized in extremis, or euthanized at terminal sacrifice. Organ weights were obtained for these parental animals and protocol-specified tissues were histologically examined. Developmental landmarks (balanopreputial separation and vaginal patency) were evaluated

on pups selected for the F_1 generation beginning on PND 25 for the females and PND 35 for the males.

Non-selected F_1 and all F_2 pups were necropsied on PND 21; selected organs from 1 pup/sex/litter were weighed. Spermatogenic endpoints (sperm motility, morphology, and numbers) were recorded for all F_0 and F_1 males. Ovarian primordial follicle counts were recorded for all F_0 females in the control and 5000 ppm groups and all F_1 females in the control and 500 ppm groups. F_2 animal litter sizes were standardized to eight pups (four/sex, where possible) on PND 4 and all surviving pups were necropsied on PND 21 and selected organs were weighed from 1 pup/sex/litter.

In order to confirm data from the 90-day rat study with DCSA (5.8/6), three additional groups of female rats (10/group) (satellite animals) were included in this study. These non-mated females received DCSA in the diet for at least 90 consecutive days at dose levels of 0, 50, and 500 ppm. Ten F_0 males from the 0, 50, and 500 ppm groups were also evaluated in conjunction with the satellite females following completion of weaning of the F_1 pups. Evaluations included weekly clinical observations and measurements of body weights and food consumption. Standard haematology and clinical chemistry parameters were evaluated. The animals received a gross necropsy examination and stomachs were evaluated histologically.

Animal assignment: Animals were randomly assigned to the test groups noted in Table 1 using a computer-based randomization procedure. The individual body weights and corresponding animal identification numbers were entered into the WIL Toxicology data management System (WTDMS^{TM}). A printout containing the animal numbers, corresponding body weights and individual group assegnments was generated based on body weight stratification randamized in a block design. The animals were then arranged into groups according to the printout. The basal diet was offered *ad libitum* to all groups throughout the acclimation period and to control group (Group 1) throughout the study. Begining study week 0, the appropriate test diet was offered to groups 2-4 for at least 70 days prior to mating and throughout the mating and post-mating periods.

TABLE 1. Animal assignment

Test group	Dose in diet (ppm)	Animals/group			
		P Males	P Females	F_1 Males	F_1 Females
Control	0	30	30	30	30
MON 52708	50	30	30	30	30
MON 52708	500	30	30	30	30
MON 52708	5000	30	30	30	30

Methods: F1 and F2 Litter Data

Litter Reduction (5.4.2)

On day 4 postpartum, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible); excess pups were weighed, euthanized by intraperitoneal injection of sodium pentobarbital and discarded on PND 4. Dead pups were examined grossly for external and internal abnormalities, and a possible cause of death was determined, if possible, for pups born or found dead. Intact offspring dying or euthanized in extremis (by intraperitoneal injection of sodium pentobarbital) from PND 0 to 4 were necropsied. Findings were recorded as either developmental variations (alterations in anatomic structure that are considered to have no significant biological effect on animal health or body conformity and/or occur at high incidence, representing slight deviations from normal) or malformations (those structural anomalies that alter general body conformity, disrupt or interfere with normal body function, or may be incompatible with life) as appropriate. A detailed gross necropsy was performed on any pup dying after PND 4 and prior to weaning; tissues were preserved in 10% neutral-buffered formalin for possible future histopathological examination only as deemed necessary by the gross findings. Carcasses of all F1 pups in the 5000 ppm group that were euthanized in extremis during PND 0-21, had a clinical finding of blackened ventral abdominal area and had dark green intestinal contents at necropsy were preserved in 10% neutral-buffered formalin for microscopic examination.

Postmortem observations:

a. Parental animals: All surviving F0 adults were euthanized following the selection of the F1 generation and completion of a detailed clinical observation. All surviving F1 adults were euthanized following weaning of the F2 pups. A complete necropsy and selective histopathologic examination were conducted on all parental animals (F0 and F1). The necropsy included examination of the external surface, all orifices, the cranial cavity, the external surfaces of the brain and spinal cord, and the thoracic, abdominal and pelvic cavities, including viscera.

Additionally, the ears and mediastinal lymph nodes were examined for all F0 animals that were necropsied on or after 22 August 2007. Clinical findings that were verified at necropsy were designated CEO (correlates with externally observed).

For F1 females that delivered or had macroscopic evidence of implantation, the numbers of former implantation sites (the attachment site of the placenta to the uterus) were recorded. The number of unaccounted-for sites was calculated for each female by subtracting the number of pups born from the number of former implantation sites observed.

b. Offspring: The F1 offspring not selected as parental animals and all F2 offspring were sacrificed at PND 21. A subset of these animals (1 pup/sex/litter) were subjected to postmortem macroscopic examinations that emphasized developmental morphology and organs of the reproductive system. For microscopic examination,

organs from F1 and F2 animals (1 pup/sex/litter were collected and preserved in 10% neutral-buffered formalin. These included brain, spleen, bone with marrow (sternebrae), stomach, ileum jejunum, thymus and all gross lesions. In addition, microscopic evaluations were performed on the following tissues from specified F1 pups in the 5000 ppm group:

- Stomach, jejunum and ileum from pups euthanized in extremis that were noted with "ventral abdominal area blackened" at the clinical observation and had an associated necropsy finding of "dark green intestinal contents"
- Skin of pups noted with "desquamation" or "uneven hair loss/hair growth"
- Gross lesions

Methods for Calculating Indices:

Reproductive indices: The following reproductive indices were calculated from breeding and parturition records of animals in the study. Mating, fertility, copulation and conception indices were calculated as follows:

$$\text{Male (Female) Mating Index (\%)} = \frac{\text{No. of Males (Females) with Evidence of Mating (or Females Confirmed Pregnant)}}{\text{Total No. of Males (Females) Used for Mating}} \times 100$$

$$\text{Male Fertility Index (\%)} = \frac{\text{No. of Males Siring a Litter}}{\text{Total No. of Males Used for Mating}} \times 100$$

$$\text{Male Copulation Index (\%)} = \frac{\text{No. of Males Siring a Litter}}{\text{No. of Males with Evidence of Mating (or Females Confirmed Pregnant)}} \times 100$$

$$\text{Female Fertility Index (\%)} = \frac{\text{No. of Females with Confirmed Pregnancy}}{\text{Total No. of Females Used for Mating}} \times 100$$

$$\text{Female Conception Index (\%)} = \frac{\text{No. of Females with Confirmed Pregnancy}}{\text{No. of Females with Evidence of Mating (or Females Confirmed Pregnant)}} \times 100$$

Offspring viability indices: Each litter was examined twice daily for survival, and all deaths were recorded. All pups were individually identified by application of tattoo markings on the digits following completion of parturition on PND 0. A daily record of litter size was maintained.

Litter parameters were defined as follows:

$$\text{Mean Live Litter Size} = \frac{\text{Total Viable Pups on PND 0}}{\text{No. Litters with Viable Pup om PND 0}}$$

$$\text{Postnatal Survival Between Birth and PND 0 or PND 4 (Pre-Selection) (\% Per Litter)} = \frac{\Sigma\,(\text{Viable Pups Per Litter on PND 0 PND 4/No. of Pups Born Per Litter})}{\text{No. of Litters Per Group}} \times 100$$

$$\text{Postnatal Survival for All Other Intervals (\% Per Litter)} = \frac{\Sigma\,(\text{Viable Pups Per Litter at End of Interval N/Viable Pups Per Litter at Start of Interval})}{\text{No. of Litters Per Group}} \times 100$$

Where N = PND 0-1, 1-4 (Pre-Selection), 4 (Post-Selection)-7, 7-14, 14-21 or 4 (Post-Selection)-21

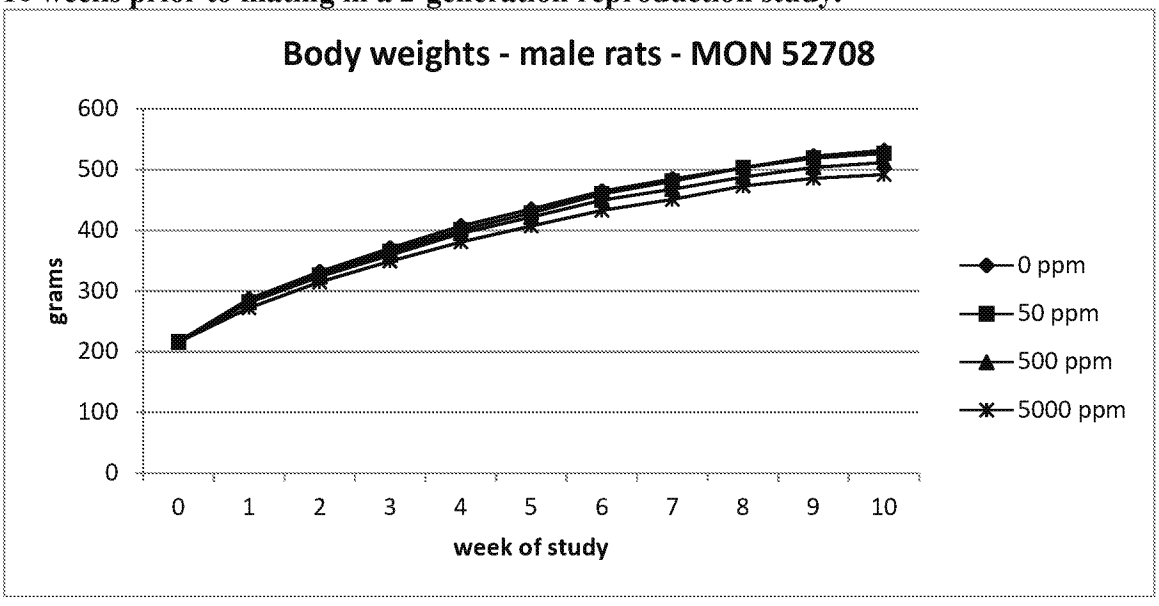
Information on viability indices calculated from lactation records of litters in the study were not included in the study methods.

II. RESULTS AND DISCUSSION

Adult animal general observations:

There were no test substance-related deaths in the F_0 or F_1 generations and there were no test substance-related clinical findings noted at the weekly examinations. Test substance-related and significantly lower mean body weight ($p<0.05$ or $p<0.01$), body weight gains, and food consumption were noted in the F_0 males and females in the 5000 ppm group (absolute body weight data shown in Figure 1 and Table 2 for study weeks 1-10. Body weights were decreased -8% and -11% for males and females, respectively, in the 5000 ppm group at week 10. No test-substance-related effects were observed on mean body weights, body weight gains, or food consumption in the F_0 50 and 500 ppm groups.

Figure 1. Body weights for F_0 male and female rats receiving DCSA in the diet for the 10 weeks prior to mating in a 2-generation reproduction study.



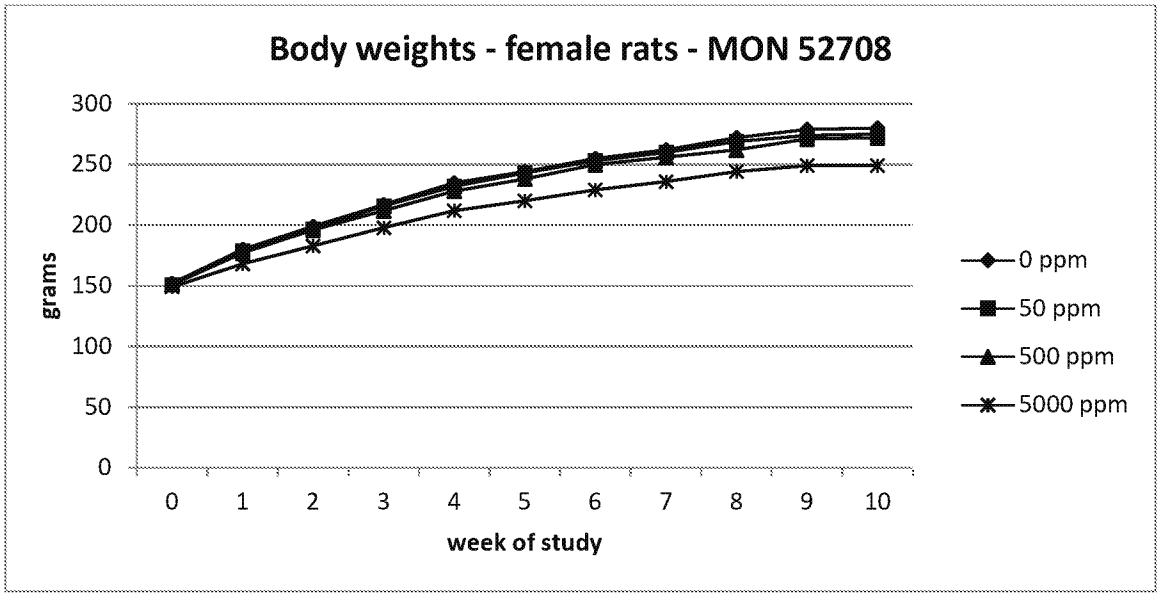


Table 2. Summary results of body weight (g), body weight changes (g) and food consumption in F0 rats in pre-mating period (study weeks 0-10)

Time	Dose Level (ppm)							
	(F0) Males				(F0) Females			
	0	50	500	5000	0	50	500	5000
Body weight (g)[a]								
Week -1	147±7.4	148±7.0	148±6.6	148±6.6	114±3.8	113±6.0	114±3.7	114±4.3
Week 0	217±10	217±10.0	216±9.7	216±9.4	152±5.6	151±5.4	151±5.5	149±5.6
Week 1	287±14	282±18	281±17	272±14**	180±7.9	179±8.5	177±10	168±7.3**
Week 3	371±23	365±25	359±26	349±23**	217±12	216±13	212±14	198±10**
Week 4	407±26	401±28	395±29	381±25**	235±14	232±15	228±15	212±10**
Week 5	435±28	429±29	422±31	407±28**	244±15	243±14	238±16	220±11**
Week 6	465±31	460±34	450±31	433±30**	255±17	253±14	250±17	229±12**
Week 7	485±34	481±39	468±32	451±34**	262±20	260±17	256±18	236±13**
Week 8	503±39	503±40	488±36	473±37**	272±19	269±19	262±20	244±14**
Week 9	522±40	519±42	504±39	486±38**	279±22	274±19	271±20	249±14**
Week 10	532±38	527±43	512±40	492±38**	280±22	275±19	272±21	249±14**
Body weight changes (g) [a]								
Week -1-0	69±6.3	69±5.3	68±6.1	68±6.2	37±5.2	38±4.8	37±5.2	35±4.9
Week 0-1	70±7.0	66±10	65±9.7	56±7.0**	28±5.3	28±5.1	27±6.3	18±4.5**
Week 1-2	45±6.9	44±7.0	43±6.6	43±5.6	19±6.2	18±3.7	19±3.4	16±5.4*
Week 2-3	39±7.1	39±6.2	35±6.0*	34±7.0**	18±6.4	19±5.1	16±5.5	15±4.4
Week 3-4	36±6.0	36±5.9	36±7.0	32±5.4*	18±5.2	17±4.4	16±5.4	14±4.3**
Week 4-5	28±8.4	28±8.8	28±6.0	26±6.5	9.0±5.5	11±5.0	11±5.2	8.0±3.7
Week 5-6	30±5.7	30±10	28±6.2	27±5.2	12±5.0	10±5.0	11±5.2	9.0±4.1
Week 6-7	20±5.5	22±8.5	18±7.0	17±7.1	6.0±6.2	6.0±6.1	6.0±5.6	6.0±3.8
Week 7-8	18±14	22±5.8	20±7.6	22±5.3	10±9.3	10±9.6	6.0±4.9	8.0±7.0
Week 8-9	19±5.1	16±6.7	16±7.1	13±6.2**	7.0±7.6	5.0±5.8	9.0±9.7	5.0±4.6
Week 9-10	10±6.2	8±5.9	8.0±5.8	6.0±5.0	1.0±7.9	1.0±6.0	1.0±5.2	0.0±6.2
Food consumption (g/animal/day) [a]								
Week -1-0	24±1.2	24±1.5	24±1.3	24±1.3	18±1.0	17±1.6	18±0.9	18±0.8
Week 0-1	28±1.5	27±2.2	27±1.9	25±1.2**	18±1.3	18±1.4	18±1.3	16±1.0**
Week 1-2	29±1.7	28±2.5	28±2.5	27±1.9*	19±1.4	19±1.5	18±1.2	17±1.2**
Week 2-3	30±2.5	29±2.5	28±2. **1	27±1.7**	20±1.8	20±1.6	19±1.4	17±1.4**
Week 3-4	30±2.0	29±2.4	29±2.1	28±1.9**	20±1.5	20±1.6	20±1.4	18±1.4**
Week 4-5	31±2.0	30±2.6	29±2.5*	28±2.0**	20±2.1	20±1.8	20±1.5	18±1.4**
Week 5-6	31±2.2	30±2.9	29±1.9**	28±2.2**	20±1.8	20±2.1	20±1.6	18±1.3**
Week 6-7	30±1.9	29±2.7	28±2.0	27±2.1**	20±2.5	19±1.8	20±1.4	18±1.4**
Week 7-8	29±2.0	29±2.7	28±2.0	28±3.4	20±1.6	19±1.9*	19±1.4**	18±1.4**
Week 8-9	30±2.3	30±2.9	28±2.3*	27±1.8**	20±2.1	19±1.8	19±1.5	18±1.4**
Week 9-10	30±2.3	30±2.4	29±2.0*	28±1.8**	20±2.4	19±1.9	20±2.0	18±1.4**

[a] Data obtained from pages 116-135 and 140-145 in the study report.

* Statistically different from control, $p<0.05$. ** $p<0.01$.

Table 3 shows the dosages on a mg/kg/day basis for various time points in the study for the F_0 and F_1 adults.

Table 3: Mean calculated test substance consumption for the F_0 and F_1 generation adults in mg/kg/day

Dietary level (ppm)	Males	Females		
	Prior to mating	Prior to mating	During gestation	During lactation
F_0 generation				
0	0	0	0	0
50	4	4	3	8
500	37	43	34	78
5000	362	414	323	610
F_1 generation				
0	0	0	0	0
50	4	5	3	8
500	41	52	34	79

Satellite adult animals:

No differences were observed when comparing control and test substance-treated satellite animals with regards to clinical observations, body weights, food consumption, and haematology, clinical chemistry, gross necropsy, and stomach microscopic evaluations.

Adult animal reproductive performance, gestational length, and parturition:

No test substance-related effects on F_0 or F_1 reproductive performance were observed at any exposure level. This included evaluations of mating, fertility, male copulation and female conception indices and number of pups born. The number of former implantation sites and the number of unaccounted sites was inadvertently not recorded and thus could not be evaluated for the F_0 generation. There was, however, no effect on the number of former implantation sites and the number of unaccounted sites for the F_1 generation.

TABLE 4. Reproductive performance [a]				
Observation	Dose group (ppm)			
	Control	50	500	5000
F_0 Generation - litter A				
Mean (±SD) precoital interval (days)	3.0±2.37	1.8±1.12*	2.5±1.55	2.0±1.07
MALES				
Number mated	30	30	30	30
Number fertile	29	28	29	29
Fertility not determined	1	2	1	1
Intercurrent deaths	1	1	0	0
FEMALES				
Number mated	30	30	30	30
Number fertile	29	28	29	29
Fertility not determined	1	2	1	1
Intercurrent deaths	0	0	0	0
Mean (±SD) gestation interval (days)	21.7±0.53	21.6±0.50	21.6±0.49	21.7±0.42
Number of litters	29	26	28	29

[a] Data obtained from pages 176-178 in the study report.
* Statistically different from control, $p<0.05$.
** Statistically different from control, $p<0.01$.

The mean number of days between pairing and coitus was comparable for control and treated groups for F_0 and F_1 generations. The mean lengths of oestrous cycles in the test substance-exposed groups were also similar to the control group values. No test substance-related effects were noted on F_0 or F_1 gestation lengths or the process of parturition.

Adult animal spermatogenic endpoint evaluations:

No test substance-related effects were observed on F_0 or F_1 spermatogenesis endpoints (mean testicular and epididymal sperm numbers and sperm production rate, motility, progressive motility, and morphology) in males at any exposure level.

Adult animal gross pathology, organ weights, and histopathology:

There were no findings of treatment-related effects of exposure to DCSA (MON 52708) based on gross examination of F_0 or F_1 animals. There were no test substance-related changes on organ weights (absolute or relative-to-body weight) in F_0 or F_1 males or females at any exposure level. In the 5000 ppm females, statistically lower absolute mean liver and ovarian weights and higher relative mean brain and kidney weights were noted compared to controls. However, these changes were considered secondary to the reduction in mean final body weight and not a direct effect of DCSA. There were no test substance-related histological changes at any exposure level in F_0 or F_1 animals. The mean number of ovarian primordial follicles in F_0 females at 5000 ppm was similar to that in the control group. Microscopic evaluation of the reproductive organs of F_0 and F_1 parental animals

suspected of reduced fertility (e.g., those that failed to mate, conceive, sire, or deliver healthy offspring or for which oestrous cyclicity or sperm number, motility, or morphology were apparently affected) did not reveal the cause of infertility.

F_1 and F_2 Generation Pups:

Litter data and postnatal survival. Test substance-related decreases in postnatal survival in the F_1 pups were observed at 5000 ppm, as shown in Tables 5 and 6. Clinical signs of toxicity observed at 5000 ppm included pale body, blackened ventral abdomens, distended abdomens, uneven hair growth, desquamation, and dark green intestinal contents. Because of the decreased postnatal survival in the F_1 5000 ppm dose group (e.g. PND 1-4 survival of 88.2% vs 99.2% in controls), clinical signs of toxicity and substantially decreased body weights, all surviving pups at 5000 ppm were terminated at weaning. The general physical condition of F_1 and F_2 pups at 50 and 500 ppm was unaffected by test diet exposure.

The mean number of F_1 and F_2 pups born, live litter size, and percentage of males per litter at birth were comparable for control and all treated groups; postnatal survival was unaffected by test diet exposure at 50 and 500 ppm.

Table 5: Multigeneration study with DCSA in the rat: postnatal survival

	F_1				**F_2**		
	0 ppm	**50 ppm**	**500 ppm**	**5000 ppm**	**0 ppm**	**50 ppm**	**500 ppm**
Postnatal survival % (PND 0, relative to # born)	98.5	99.0	98.7	96.4	98.9	98.1	99.6
Postnatal survival % (PND 0-1)	99.3	99.3	98.6	94.9	98.5	99.8	99.2
Postnatal survival % (PND 1-4, pre-selection)	99.2	98.7	99.6	88.2	99.6	99.1	98.2
Postnatal survival % (PND 4 (post-selection)-7	100.0	100.0	98.7	99.5	100.0	99.6	99.5
Postnatal survival % (PND 7-14)	100.0	100.0	99.6	91.3	99.4	99.5	99.5
Postnatal survival % (PND 14-21)	100.0	100.0	99.6	72.6**	99.0	99.6	100.0

** = $p \leq 0.01$.
Shown on pages 225, 225, 358, 359

Table 6. Summary of postnatal data

TABLE 7. Litter parameters for F_1 and F_2 generations [a]				
Observation	Dose group (ppm)			
	0	50	500	5000
F_1 Generation				
Mean implantation sites	Not Recorded	NR	NR	NR
Number born	14.0±1.81	14.0±2.85	14.7±2.88	13.3±2.47
Sex ratio day 0 (% %)	50±13.77	50.8±13.23	50.9±13.42	50.2±15.23
Mean litter size Day 0	13.8±1.68	13.8±2.87	14.5±2.85	12.9±3.0
Viability index	98.5±4.1	99.0±3.97	98.7±3.12	96.4±12.47
F_2 Generation				
Mean implantation sites	15±2.57	13.7±3.61	14.4±3.68	NA
Number born	14.2±2.35	13.2±3.87	13.9±3.52	NA
Sex ratio day 0 (% %)	47.6±13.58	55.3±16.05	49.6±12.93	NA
Mean litter size Day	14.1±2.40	12.9±3.81	13.8±3.46	NA
Viability index	98.9±3.36	98.1±7.01	99.6±1.57	NA

[a] Data obtained from pages 224, 235, 358 and 359 in the study report.
[b] Before standardization (culling)
[c] After standardization (culling)
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$

Pup weights. Test substance-related decreases were observed in F_1 male and female pup body weights (Table 7) and body weight gains (data not shown) at 5000 ppm. Mean F_1 male and female body weights were significantly decreased ($p \leq 0.01$) at PND1 for 5000 ppm animals compared to controls. In addition, mean F_1 male and female pup body weight gains at 5000 ppm were significantly lower than controls throughout the entire pre-weaning period, resulting in significantly lower mean body weights on PND 4, 7, 14, and 21.

At 500 ppm, mean F_1 male and female pup body weights on postnatal days 14 and 21 were reduced approximately -6% to -9% of controls; female pup body weight was also reduced at week 18 (-7%).

Table 7: Body weights[a] for F_1 and F_2 pups from 2-generation reproduction study with DCSA.

Day on study	**F_1 control**	F_1 50 ppm	F_1 500 ppm	F_1 5000 ppm		**F_2 control**	F_2 50 ppm	F_2 500 ppm	F_2 5000 ppm
Males									
PND1	6.8 ±0.6	6.9 ±0.6	6.8 ±0.6	**6.2** ±0.7**		6.9 ±0.5	7.0 ±06	7.0 ±0.9	ND
PND4	9.7 ±1.0	9.8 ±1.2	9.5 ±0.9	**8.2** ±1.2**		10.0 ±1.1	10.4 ±1.1	10.2 ±1.7	ND
PND7	15.8 ±1.4	15.9 ±1.5	14.9 ±2.2	11.4 ±2.2		15.9 ±1.9	16.4 ±1.7	16.0 ±2.1	ND
PND14	31.6 ±2.1	31.6 ±2.6	**29.4** ±2.8*	**16.7** ±4.2**		30.9 ±4.4	32.1 ±2.7	31.5 ±3.5	ND
PND21	49.7 ±4.5	49.7 ±4.5	**45.3** ±4.3**	**23.4** ±6.1**		46.2 ±5.7	48.1 ±4.9	46.9 ±5.5	ND
Wk 17	79 ±6	82 ±10	75 ±8	ND		ND	ND	ND	ND
Wk 18	125 ±18	131 ±17	119 ±16	ND		ND	ND	ND	ND
Wk 19	176 ±32	187 ±23	172 ±19	ND		ND	ND	ND	ND
Wk 20	241 ±35	248 ±30	233 ±24	ND		ND	ND	ND	ND
Wk 21	299 ±34	306 ±35	292 ±28	ND		ND	ND	ND	ND
Females									
PND1	6.5 ±0.7	6.5 ±0.6	6.4 ±0.6	**5.8** ±0.8**		6.5 ±0.6	6.6 ±0.7	6.6 ±0.9	ND
PND4	9.2 ±1.1	9.3 ±1.1	9.0 ±1.0	**7.6** ±1.2**		9.4 ±1.1	9.8 ±1.3	9.7 ±1.9	ND
PND7	15.2 ±1.6	15.0 ±1.6	14.2 ±2.4	**10.7** ±2.0**		14.9 ±1.7	15.6 ±1.8	15.3 ±2.7	ND
PND14	30.5 ±2.5	30.6 ±2.5	**28.5** ±3.5*	**15.6** ±3.5**		29.2 ±4.0	30.8 ±3.1	30.5 ±3.7	ND
PND21	47.8 ±4.9	47.3 ±4.5	**43.6** ±5.0**	**21.2** ±4.8**		43.4 ±5.8	46.2 ±5.7	45.3 ±5.5	ND
Wk 17	73 ±5	73 ±7	69 ±8	ND		ND	ND	ND	ND
Wk 18	112 ±14	113 ±11	**104** ±14*	ND		ND	ND	ND	ND
Wk 19	146 ±19	146 ±12	140 ±14	ND		ND	ND	ND	ND
Wk 20	177 ±18	177 ±13	170 ±16	ND		ND	ND	ND	ND
Wk 21	203 ±18	202 ±16	193 ±19	ND		ND	ND	ND	ND

a – all values for DCSA are the mean±SD. * = $p \leq 0.05$, ** = $p \leq 0.01$.
PND = post natal day, Wk = week, ND = no data in F_1 generation because these pups were sacrificed at PND 21 due to high pup mortality in this dose group; the F_2 generation had scheduled termination of all groups at weaning (PND21).

Organ weights. Mean absolute and relative (to final body weight) thymus weights in F_1 males and females at 500 and 5000 ppm were significantly lower than the control group values (Table 8), however, there were no histological correlates. No other effects on absolute or relative (to final body weight) organ weights were noted in the F_1 or F_2 pups at any exposure level that were considered test substance related.

Table 8. F_1 and F_2 Pup Mean Absolute Thymus Weights (mean + SD)

F1 Pups				
	0 ppm	**50 ppm**	**500 ppm**	**5000 ppm**
Males	**0.2029 ± 0.04607**	**0.1942 ± 0.03141**	**0.1662 ** ± 0.03693**	**0.0414 ** ± 0.02631**
Females	**0.2053 ± 0.05079**	**0.1953 ± 0.03265**	**0.1784 ** ± 0.05157**	**0.0359 ** ± 0.01794**
F2 Pups				
Males	**0.1689 ± 0.03254**	**0.1729 ±0.0416**	**0.1768 ± 0.04065**	**NA**
Females	**0.1710 ± 0.03976**	**0.1885 ± 0.04326**	**0.1882 ± 0.03134**	**NA**

Table 54 on pages 236 - 237 and Table 114 pages 370-371.
**Significantly different from the control group at 0.01 using Dunnet's test

Histopathology. At 5000 ppm, hyperkeratosis of the skin was observed in both male and female pups that had gross skin lesions at the scheduled necropsy on PND 21. Hyperkeratosis of the skin was also observed in some pups at 5000 ppm in animals that had skin collected because they had clinical signs of desquamation or uneven hair loss/hair growth (Table 9). No test substance-related microscopic changes were observed from sections of the stomach, jejunum, and ileum that were collected from 5000 ppm pups that were euthanized in extremis with clinical signs of ventral abdominal area blackened and an associated necropsy finding of dark green intestinal content.

Table 9. Skin Hyperkeratosis in F1 Pups at sacrificed (PND 21) in 5000 ppm Dose Group

F1 Pups Selected for Organ Weights				
	Males		Females	
	0 ppm	5000 ppm	0 ppm	5000 ppm
Total number examined	0	4	0	5
Examined, unremarkable		0		0
Hyperkeratosis		4		5
Minimal		2		3
Mild		2		2
F1 pups euthanized in extremis or with dermal findings				
Total number examined	0	11	0	11
Examined, unremarkable		0		0
Hyperkeratosis		11		11
Minimal		3		1
Mild		7		8
Moderate		1		2

Tables 56 and 57 page 240, 242, 245, 246.

Developmental landmarks. In F_1 animals, the mean ages of attainment of balanopreputial separation and mean body weights on the day of attainment were unaffected by test diet exposure. Mean ages of attainment of vaginal patency and mean body weights were also unaffected by test diet exposure.

VI. EVALUATION, SUMMARY AND CONCLUSIONS BY REGULATORY AUTHORITY

G. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U.S. EPA

H. REVIEWER'S COMMENTS:

The endpoint for offspring toxicity, reduced body weight in F1 pups, may be a threshold event at 500 ppm dietary concentration. The reduced body weight was statistically significant only on postnatal days 14 and 21 (both sexes) and week 18 (females only). The magnitude of the decrease was minor (-6% to -9%) and was only noted in the F1 generation; pup body weights in the F2 generations were similar to controls.

Furthermore, the dose on a mg/kg/day basis may be underestimated because compound intake was calculated from the pre-mating period when food consumption is relatively low. Maternal compound intake was much greater during the period when the weight loss occurred and pups were beginning to consume feed towards the end of lactation when the body weights were decreased.

RELIABILITY RATING: Reliable (Acceptable/Guideline). This study is fully compliant with OECD.408.

DEFICIENCIES

The high dose of 5000 ppm was excessive and did not allow for a second generation for this group.

Information on viability indices calculated from lactation records of litters in the study was not included in the study report. Viability was calculated from litter parameters. Each litter was examined twice daily for survival, and all deaths were recorded. All pups were individually identified by application of tattoo markings on the digits following completion of parturition on PND 0. A daily record of litter size was maintained. This method of determining viability of the pups is valid.

All F0 animals that were necropsied on 23 or 24 August 2007 did not receive a detailed physical examination prior to necropsy.

The number of former implantation sites was not recorded for any F0 female at the time of necropsy.

These deficiencies did not interfere with validity or interpretation of the data.

Revised by U.S. Environmental Protection Agency

Study type: **Dietary Combined Chronic Toxicity/Carcinogenicity Study of MON 52708 in Rats**

Report: IIA 5.8/15. Kirkpatrick, J.B. (2009d). A 24-Month Oral (Diet) Combined Chronic Toxicity/Carcinogenicity Study of MON 52708 in Rats: 12-Month Chronic Toxicity Study. WIL Research Laboratories, LLC, unpublished report WI-2008-006/WIL-50349, MRID 47899516.

IIA 5.8/22. Kirkpatrick, J.B. (2011). A 24-month oral (diet) combined chronic toxicity/carcinogenicity study of MON 52708 in rats: Carcinogenicity phase. WIL Research Laboratories, LLC, unpublished report WI-2008-006/WIL-50349. MRID 48358003.

Dates of Work: January 18, 2008 – February 15, 2009

Guidelines: OECD 451 and 452
EPA OPPTS 870.4300
Deviations: None.
PMRA DACO 4.4.1

GLP: Yes Signed and dated GLP, Data Confidential Statements and Flagging Statement were provided.

Executive Summary:

In this combined chronic toxicity/carcinogenicity study (MRID 47899516, chronic toxicity and MRID 48358003, carcinogenicity), Sprague Dawley (Crl:CD®[SD]) rats were exposed to DCSA (MON 52708 purity 97.4% - 97.7%; Lot/batch no GLP-0603-16958-T) in the diet. Dietary concentrations were 0, 10, 100, 300, 1000 or 3000 ppm. Doses for the chronic toxicity phase were 0.6, 5.6, 16.9, 56.9, and 171.2 mg/kg/day for males and 0, 0.7, 6.9, 20.5, 68.2, and 206.2 mg/kg/day for females. Doses for the carcinogenicity phase were 0.5, 5.0, 14.6, 48.8, and 150.1 mg/kg/day in males and 0.6, 6.1, 18.4, 60.9, and 181.5 mg/kg/day in females. There were 50 male and 50 female rats in the 24 month carcinogenicity study and 20 male and 20 female rats in the 12 month chronic toxicity study.

All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed weekly. Individual body weights and food consumption were recorded at least weekly for the first 13 weeks of the study, and at least once every four weeks thereafter. Ophthalmic examinations were performed during study weeks 2 and 51. Clinical pathology parameters were evaluated for the last 10 surviving animals/sex/group: hematology and serum chemistry were evaluated during study weeks 12 and 25, and at the scheduled

necropsy (study week 52); coagulation parameters were evaluated only at the scheduled necropsy (study week 52); and urinalysis parameters were analyzed during study week 25 and at the scheduled necropsy (study week 52). Complete necropsies were conducted on all animals, and selected organs were weighed at the scheduled necropsy. Selected tissues were examined microscopically from animals in the control and 3000 ppm groups. Tissue masses (when present), pituitary glands, and gross lesions (when present) were examined from all animals.

There were no toxicologically significant treatment related effects on mortality, clinical signs, body weight, food consumption, ophthalmology, clinical chemistry, hematology, coagulation, urinalysis, or organ weights. There were no toxicologically significant effects noted for gross or microscopic pathology.

No significant toxicity occurred in this study and the NOAEL is 150 mg/kg/day, (1000 ppm dietary concentration) the highest dose tested. A LOAEL was not determined. This study is classified acceptable/non-guideline.

I. MATERIALS AND METHODS

A. Materials

1. **Test material:**	DCSA (MON 52708);
Description:	White powder
Lot/Batch#:	GLP-0603-16958-T
Purity:	97.4 to 97.9%
CAS #:	Not given
Compound Stability:	The test substance was considered stable at room temperature
Structure:	Not available

2. **Test animals**		
Species:	Rat	
Strain:	Sprague-Dawley, Crl:CD® (SD)	
Age/ weight at study initiation:	Approximately 7 weeks old at the initiation of dose administration. Male: 186 – 274 g Females: 145 – 211 g	
Source:	Charles River Laboratories, Inc., Raleigh, NC	
Housing	Individually in clean, stainless steel, wired-mesh cages suspended above cage-board.	
Diet:	Certified Rodent Lab-Diet® 5002 (PMI Nutrition International, LLC), *ad libitum*. The feed batch was analyzed for contaminants. No unacceptable levels of contaminants were present.	
Water:	Reverse osmosis-purified (on site) drinking water, *ad libitum*	
Environmental conditions:	**Temperature:**	22°C ± 3°C
	Humidity:	50 ± 20%
	Air changes:	At least 10/hr
	Photoperiod:	12 hrs Dark /12hrs light
Acclimation period:	14 days	

3. **Dose selection rationale:**

The registrant reported that the highest dose selected in this study, 3000 ppm, was slightly higher than that used in the combined chronic toxicity/carcinogenicity study performed with MON 52708's parent molecule, dicamba (2500 ppm). Dose selection

was based on results from a 13-week oral toxicity study of MON 52708 (MRID 47899507), and on a pharmacokinetic study (MRID 47899503).

In the 13-week oral toxicity study, rats were administered MON 52708 at dietary concentrations of 500, 3000, 6000, and 12,000 ppm (approximately 32, 195, 362, and 659 mg/kg/day for males and 37, 222, 436, and 719 mg/kg/day for females). Toxicity at 12,000 ppm, upon which the LOAEL was based, included substantially decreased body weights, increased liver enzymes, and decreased hematological parameters.

In the pharmacokinetic study (MRID 47899503), non-linearity of blood levels, indicative of a change in pharmacokinetic behavior occurred between approximately 125 to 250 mg/kg/day. In that study, the relationship between 24 hour plasma total radioactivity concentration values were proportional to dose for the 42 and 125 mg/kg dose levels. However, for a 12x increase in dose from from 42 to 500 mg/kg, the 24 hour concentration values increased 122x for males and 295x for females.

4. Dose preparation and analysis:

Formulations were prepared approximately weekly by mixing appropriate amounts of test substance with Rodent LabDiet #5002 (meal) and were stored at room temperature. Prior to the start of the study, stability of the test substance was assessed at room temperature for 8 and 15 days and frozen at approximately -20°C for 15, 35, 57, and 367 days. Homogeneity (top, middle, and bottom) was evaluated in the 10 and 100 ppm dosing formulations. Samples for concentration analysis were collected weekly from the middle stratum of each dosing formulation, including the control group, and were analyzed weekly for the first three weeks of the study and approximately monthly thereafter.

B. Study Design

Groups of 20 male and 20 female Sprague-Dawley (Crl:CD[SD]) rats were assigned to the chronic toxicity (12-month) phase of the study and groups of 50 males and 50 females were assigned to the carcinogenicity (24-month) phase of the study as shown in Table 1.

Table 1. Study design

Group Number	Treatment	Dose Level (ppm)	Number of Chronic Toxicity Phase Rats		Number of Carcinoginicty Phase Rats	
			Males	Females	Males	Females
1	Basal Diet	0	20	20	50	50
2	MON 52708	10	20	20	50	50
3	MON 52708	100	20	20	50	50
4	MON 52708	300	20	20	50	50
5	MON 52708	1000	20	20	50	50

The rats were checked twice daily for signs of mortality and moribundity and once weekly for signs of general toxicity. Body weights and food consumption were recorded weekly for the first 13 weeks of the study and once every four weeks thereafter. Haematology, clinical chemistry and urine analyses were performed on 10 animals/sex/group at study weeks 12, 25, and 52. Ophthalmological examinations were performed pretest at week -2 and at week 51. Standard gross pathology and histopathology examinations were also performed.

II. RESULTS AND DISCUSSION

Intake of test article: Overall mean compound consumption (on a mg/kg/day basis) was as follows (Table 2):

Table 2: Test material intake of DCSA

Dietary Conc. (ppm)	Chronic Phase (mg/kg/day)		Carcinogenicity Phase (mg/kg/day)	
	Males	Females	Males	Females
0	0	0	0	0
10	0.6	0.7	0.5	0.6
100	5.6	6.9	5.0	6.1
300	16.9	20.5	14.6	18.4
1000	56.9	68.2	48.8	60.9
3000	171.2	206.2	150.1	181.5

Food consumption was similar across all dose groups, including controls.

Clinical signs and mortality:

Survival was comparable between treated and control groups through study termination. There were no clinical signs observed in this study that suggested an effect related to treatment with DCSA.

Body weight:

Body weight values for males and females are presented in Tables 3 and 4. There were no statistically significant differences in the mean body weights compared to controls.

TABLE 3. Summary results of body weights (g) in Sprague-Dawley rats exposed daily to dietary doses of MON 52708 for 12 months (Chronic toxicity phase)

TIme	0 ppm	10 ppm	100 ppm	300 ppm	1000 ppm	3000 ppm
Males [a]						
Week 0	237±14.2	238±15.7	233±13.7	234±10.2	233±12.2	225±18.9
Week 1	282±18.1	283±19.3	279±20.1	284±14.5	279±15.8	273±22.8
Week 2	318±23.3	324±23.7	317±25.7	323±20.5	316±22.9	308±30.5
Week 3	348±30.2	358±28.3	353±30.4	357±23.4	348±26.9	340±36.2
Week 4	375±34.4	384±32.9	380±32.4	383±26.0	371±32.5	363±36.8
Week 8	461±48.1	475±46.7	476±41.6	475±40.4	462±44.4	449±58.9
Week 12	495±56.0	520±62.7	521±52.9	517±51.2	501±48.6	500±71.9
Week 32	623±70.4	654±84.3	648±77.1	645±80.3	647±62.8	634±79.8
Week 52	702±95.4	715±87.8	716±77.9	719±100.4	712±79.5	710±103
Females [a]						
Week 0	178±13.2	174±12.1	174±11.3	174±11.2	173±12.0	177±13.9
Week 1	195±18.0	190±13.1	188±13.1	193±17.0	191±15.6	190±18.5
Week 2	215±20.5	209±15.8	210±14.5	212±20.1	211±18.8	212±21.9
Week 3	229±21.3	224±19.3	224±17.7	229±22.4	226±20.5	227±25.1
Week 4	241±22.3	234±21.2	236±19.6	241±23.6	237±23.6	239±29.3
Week 8	276±26.1	272±27.9	271±26.9	279±32.1	271±27.3	275±33.2
Week 12	285±29.0	286±28.5	282±27.2	292±35.4	284±33.4	290±36.8
Week 32	349±26.9	350±44.7	341±47.8	356±68.8	333±38.7	340±54.0
Week 52	402±40.3	398±72.5	383±63.9	405±106	385±63.3	404±67.3

[a] = Mean±SD Data were obtained from results in Tables 9 and 10 on pages 101-114 of the study report

TABLE 4. Summary results of body weights (g) in Sprague-Dawley rats exposed daily to dietary doses of MON 52708 for 24 months (Carcinogenicity phase)

TIme	0 ppm	10 ppm	100 ppm	300 ppm	1000 ppm	3000 ppm
Males [a]						
Week 0	237±13.0	234±11.8	236±13.9	235±14.8	233±14.3	232±13.7
Week 24	618±73.2	605±62.3	609±58.0	635±83.9	626±66.1	611±61.2
Week 48	719±94.1	688±90.3	675±84.7	730±112.7	709±90.7	708±79.3
Week 72	751±121.7	722±124.1	737±82.4	759±146.8	742±96.1	729±105.8
Week 104	736±109.8	716±117.0	764±195.9	754±144.5	671±164.8	770±116.7
Females [a]						
Week 0	176±10.9	177±10.2	177±10.1	175±10.1	176±11.9	176±9.7
Week 24	32.0±30.3	330±34.5	334±40.2	330±37.9	334±39.4	324±32.6
Week 48	377±57.2	395±61.8	395±62.3	389±64.9	395±59.5	379±60.6
Week 72	437±67.9	446±88.9	437±95.7	437±89.8	446±76.5	444±79.7
Week 104	462±124.3	413±101.9	429±81.5	441±104.4	449±109.2	437±91.5

[a] = Mean±SD Data were obtained from Tables S25 and S26 on pages 211-234 of the study report

Haematology, clinical chemistry, and urinalysis:

There were no adverse treatment-related effects shown in clinical chemistry, haematology, coagulation, and urinalysis testing. Lower total bilirubin was noted in the 3000 ppm group males and in the 300, 1000, and 3000 ppm group females compared to concurrent controls at study week 52, although this effect is not considered adverse. Exposure to salicylates may result in hypobilirubinemia due to altered protein binding through dissociation of albumin and bilirubin in blood plasma. DCSA is a chlorinated salicylate and thus the decreased bilirubin levels noted in this study may be a consequence of altered protein binding.

Ophthalmology

There was no evidence of test-substance-related adverse findings on the eyes following ophthalmological examinations.

Organ weights:

There were no statistically significant differences in final body weights or organ weights when the control and test substance-treated groups were compared.

Gross pathology:

There were no test substance-related macroscopic findings in either the chronic or carcinogenicity phases. In the chronic toxicity phase, several groups had depressed areas of

the kidneys which generally correlated microscopically with chronic progressive nephropathy, a common age-related finding in toxicity studies.

Histopathology:

There were no adverse treatment-related effects found on microscopic examination.

In the chronic toxicity phase, there was a slightly higher incidence of pituitary pars distalis adenomas in the 3000 ppm males (6/20) compared to control males (3/20), but the incidence was not dose related, was not statistically significant, and was not increased in the carcinogenicity phase (Tables 5 and 6).

In the chronic toxicity phase, the 3000 ppm group females had a slightly higher incidence of mammary gland fibroadenomas and uterine endometrial stromal polyps compared to the concurrent control group (3/20 versus 1/20 for both lesions), but the differences were not statistically significant and were not increased in the carcinogenicity phase.

Table 5. Incidence of Selected Microscopic Observations (12-month Toxicity Phase)												
	Males						Females					
Dose (ppm)	0	10	100	300	1000	3000	0	10	100	300	1000	3000
Pituitary [a]	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**
Adenoma, pars	3	2	7	4	5	6	7	7	2	4	2	9

[a] = Number of tissues examined from combined intervals in bold.

Table 6. Incidence of Selected Microscopic Observations (24-month Carcinogenicity Phase) All Animals: Unscheduled deaths + scheduled necropsy.												
	Males						Females					
Dose (ppm)	0	10	100	300	1000	3000	0	10	100	300	1000	3000
Liver [a]	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**34**	**33**	**35**	**36**	**50**
Adenoma, hepatocellular	1	1	4	1	1	3	0	0	0	1	0	0
Congestion	10	15	15	22*	19	17	3	9	4	1	6	7
Pituitary [a]	**50**	**40**	**44**	**45**	**41**	**50**	**50**	**45**	**48**	**44**	**47**	**50**
Adenoma, pars	35	28	27	31	27	34	42	43	45	41	44	41
Lymph Node, Mediastinal [a]	**50**	**29**	**34**	**38**	**36**	**49**	**46**	**28**	**28**	**27**	**29**	**45**
Macrophages, pigmented	10	3	4	8	4	13	19	10	10	9	16	30*
Nasal Cavity (Level III) [a]	**50**	**31**	**34**	**40**	**36**	**50**	**49**	**29**	**31**	**29**	**27**	**50**
Inflammation, acute	0	3	2	5	3	7*	2	5	5	6	4	3
Ovary [a]	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**50**	**50**	**50**	**50**	**50**	**50**
Cysts	-	-	-	-	-	-	13	18	15	17	11	16
Spleen [a]	**50**	**35**	**37**	**41**	**37**	**50**	**50**	**29**	**34**	**31**	**28**	**50**
Macrophages, pigmented	14	4	13	13	9	15	23	15	18	16	14	35*
Uterus [a]	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**50**	**50**	**50**	**50**	**50**	**50**
Hyperplasia, cystic endometrial	-	-	-	-	-	-	18	12	16	6	21	25
Polyp	-	-	-	-	-	-	2	4	4	2	9	6

[a] = Number of tissues examined from combined intervals in bold.
* = Statistically significant, $p \leq 0.05$ NA = Not applicable; gender specific

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U.S. EPA

B. REVIEWER'S COMMENTS:

No significant toxicity occurred in either the chronic toxicity phase or the carcinogenicity phase and the NOAEL is 150 mg/kg/day (1000 ppm dietary concentration), the highest dose tested. The registrant proposed that dosing was adequate because there was evidence of saturation of metabolism. However, regardless of saturation, the animals could have tolerated a larger dose because only minimal toxicity occurred in the 90-day rat study (MRID 47899507) at 12000 ppm. At least it was demonstrated that there was not a significant increase in toxicity with increased duration of exposure. Because no toxicity occurred, the study is classified acceptable/non-guideline.

Revised by U.S. Environmental Protection Agency

***In vitro* Bacterial Gene Mutation (Salmonella typhimurium)/ mammalian activation gene mutation assay**

Report: IIA 5.8/20 Mecchi, M. (2006). Salmonella - Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay with MON 52708. Covance Laboratories Inc. Vienna, Virginia; Study CV-2006-05428 September, 2006, unpublished. MRID 47899509

Guidelines: OPPTS 870.5100 (1998)
OECD 471 (1997)
JMAFF (Shirasu, 1988)
PMRA DACO 4.5.4

GLP: YES OECD Principles of GLP, ENV/MC/CHEM (1998) 17
Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

GLP
Exceptions: None

EXECUTIVE SUMMARY:
In independent trials of a reverse gene mutation assay (MRID 47899509), MON 52708 (Purity 97.9%; Lot No. GLP-0603-16958-T), prepared in dimethyl sulfoxide (DMSO) was tested in 4 strains of Salmonella typhimurium (TA100, TA98, TA1535 and TA1537) and in E. coli WP2uvrA at 6 concentrations ranging from 33.3 to 5000 µg/plate with or without S9 activation. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Aroclor™ 1254.

Concentrations for the main assay were determined from the results of a dose range-finding study with S. typhimurium TA100 and the E. coli strain in which 10 levels in the range of 6.67 - 5000 µg/plate with or without metabolic activation were assayed. Following incubation for 52± 4 hours, growth inhibition, as indicated by either a reduction in the background lawn of growth or a reduction in the number of revertant colonies was not observed in the Salmonella or E. coli strains until 3300 µg/plate without S9. The S9-activated test material was not cytotoxic. Based on the above findings, the highest concentration selected for the two trials was set at 5000 µg/plate +/- S9. Growth inhibition in the number of revertant colonies and /or the background Lawn was observed in all Salmonella and the E.coli strains at 5000 µg/plate –S9 and in the majority of strains at at 2500 µg/plate –S9. The test material was not cytotoxic in the presence of S9 activation at any concentration. However, the mean numbers of revertant colonies for all strains were not appreciably increased by treatment with the test substance at all levels, with and without S9. The numbers of revertant colonies in all strain-specific positive control groups were clearly increased and were within the laboratory historical control ranges.

Under the conditions of this study, MON 52708 did not induce gene mutation, either with or without metabolic activation, in any of the Salmonella or the E. coli strains up to concentrations that were cytotoxic or the limit dose for this test system.

The study is classified as **reliable (acceptable/guideline)** and satisfies the guideline requirements (OCSPP 870.5100; OECD 471) for in vitro mutagenicity (bacterial reverse gene mutation) data.

IIIA 7.1.1 I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Description:	White solid
Lot/Batch#:	GLP-0603- 16958-T
Purity:	97.9%
CAS #:	Not given
Stability of test compound:	Listed with an expiration date of March 20, 2007.
Solvent:	Dimethyl sulfoxide (DMSO)
Structure:	None given in the report

2. Control Materials

Negative:	None		
Solvent/concentration	DMSO/0.05mL		
Positive			
Nonactivation	2-nitrofluorene	1.0 µg/plate	TA98
	ICR-191	2.0 ug/plate	TA1537
	Sodium azide; NaN_3	2.0 µg/plate	TA100, TA1535
	4-nitroquinoline-N-oxide	1.0 µg/plate	WP2uvrA
Activated	Benzo(a)pyrene; B(a)P	2.5. µg/plate	TA98
	2-Aminoanthracene: 2-AA	2.5 µg/plate	TA1535, TA100,TA1537
		25.0 µg/plate	E. coli WP2uvrA

3. Activation: S9 derived from male Sprague-Dawley rats

X	Induced	X	Aroclor 1254	X	Rat	X	Liver
	Non-induced		Phenobarbital		Mouse		Lung
			β-naphthoflavone		Hamster		Other (name)
			None		Other (name)		

The S9 fraction was prepared commercially by Molecular Toxicology, Inc (Lot Nos. 1963 and 2001 with 40.2 and 40.5 mg protein/mL, respectively). The S9 fraction (1 ml) was added to a cofactor mix, which contained the following components:

S9 Mix Components

Component	Amount
H2O	0.70 mL
1M NaH2PO4/Na2HPO4, pH 7.4	0.10 mL
0.25M Glucose-6-phosphate	0.02 mL
0.10M NADP	0.04 mL
0.825M KCl/0.2M MgCl2	0.04 mL
S9 Homogenate	0.10 mL

The final S9 culture concentration was approximately 10%.

4. Test organisms:

	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537		TA1538	X	WP2uvrA		WP2

Properly maintained?	X	Yes		No
Checked for appropriate genetic markers (rfa mutation, R factor)?	X	Yes		No

5. Test compound concentrations preparation/used:

MON 52708 and the positive control substances were dissolved in DMSO. The test substance was weighed, dissolved in the requisite volume of solvent to prepare the test solutions at the highest soluble concentration (100 mg/ml). Test solutions at the lower levels were prepared by serial stepwise dilution. Test solutions were prepared at the time of use.

Dose range-finding cytotoxicity assay: Ten doses ranging from 6.67 to 5000 ug/plate were tested; single plates/concentratrion/strain/condidiiton were used with S. typhimurium TA100 and E. coli WP2uvrA

Mutagenicity assay:
Non-activated conditions: 0, 33.3, 100, 333, 1000, 2500 and 5000 µg/plate (both trials)

Activated conditions: 0, 33.3, 100, 333, 1000, 2500, 5000 µg/plate (both trials)

In both trials of the mutagenicity assay, triplicate plates were prepared per strain for all test article concentrations, solvent, and positive controls in the presence and absence of S9-activation.

B. STUDY DESIGN AND METHODS
1. In life (experimentation) dates: April 27, 2006 – June 09, 2006

2. Preliminary cytotoxicity assay:
Concentrations for the main assay were determined from the results of the preliminary cytotoxicity test, which was performed as described below for the plate incorporation mutation assay.

3. Mutation assay:
When S9 mix was not required, 100 µL of tester strain and 50 µL of vehicle, positive control or dilution of the test article dose were added to 2.5 mL of molten selective top agar (maintained at 45 ± 2°C). When S9 mix was required, 500 µL of S9 mix, 100 µL of tester strain and 50 µL of vehicle, positive control or test article dilution were added to 2.0 mL of molten selective top agar. After the required components had been added, the mixture was vortexed and overlaid onto the surface of 25 mL of minimal bottom agar contained in a 15 x 100 mm petri dish. After the overlay solidified, the plates were inverted and incubated for 52 ± 4 hours at 37 ± 2°C. Positive control articles were plated using a 50 µL plating aliquot.

4. Statistical analysis:

The data was not subjected to statistical analysis; but means and standard deviations were calculated.

5. Evaluation criteria:

The test article was considered to be positive for mutagenicity if the number of revertant colonies of any strain were increased by more that twice the solvent control, and the response was dose-dependent and reproducible for strains TA98, TA100, and WP2uvrA and at least a 3-fold increase in the mean revertants per plate of at least one of the TA1535, TA1537 tester strains.

NOTE: Historical control data for the solvent and positive controls (using the plate incorporation method) were presented by the performing laboratory.

IIIA 7.1.2 II. RESULTS AND DISCUSSION

A. Preliminary cytotoxicity assay: Data from the dose range-finding study are presented in study report Table 1. As shown, cytotoxicity, indicated by either a reduction in the background lawn of growth and/or a reduction in revertant colonies was observed in S. typhimurium TA100 strain and the E. coli strain at ≥ 3330 µg/plate -S9. The S9-activated test material was not cytotoxic for any strain. Compound precipitation was not seen at any concentration.

B. Mutation assay: On the basis of the preliminary cytotoxicity data, the highest concentration used for the two trials of the mutation assay main assays was 5000 µg/plate +/-S9. Summarized results form both trials are presented in study report Tables 3 and 5. In agreement with the preliminary results, cytotoxicity was noted at 5000 µg/plate –S9 in all strains and for the majority of strain at 2500 µg/plate –S9. Similarly, the S9 activated test material was not cytotoxic at any concentration. These findings were confirmed in Trial 2 (Table 5). By contrast, the numbers of

revertant colonies in all strain-specific positive control groups were increased. The numbers of revertant colonies in the solvent and all positive control groups were within the laboratory historical control range.

IIIA 8 III. EVALUATION, SUMMARY AND CONCLUSIONS BY REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:

RELIABILITY RATING:
Totally reliable.
This study is compliant with OECD 471(1997)

C. CONCLUSIONS:

MON 52708 was tested up to cytotoxic and/or precipitating concentrations (5000 µg/plate) in all strains in both trials in the presence and absence of S9-activation. There were no treatment-related increases in the mean number of revertants/plate in any strain (+/-S9). The positive controls induced marked increases in revertant colonies compared to controls in all strains in the presence and absence of S9-activation.

Accordingly, MON 52708 is negative in this test system in a well-done study.

Table 1: Dose Rangefinding Study

Test Article ID: MON 52708

Assay No.: 28296-0-409OECD Trial No.: A1

Date Plated: 04-May-06 Vehicle: DMSO

Date Counted: 08-May-06 Plating Aliquot: 50 µL

		Revertants per Plate			
Dose/Plate		TA100	Background Lawn[a]	WP2uvrA	Background Lawn[a]
Microsomes: Rat Liver					
Vehicle Control		117	N	18	N
Test Article	6.67 µg	116	N	11	N
	10.0 µg	128	N	20	N
	33.3 µg	120	N	24	N
	66.7 µg	138	N	15	N
	100 µg	125	N	10	N
	333 µg	111	N	11	N
	667 µg	139	N	18	N
	1000 µg	122	N	16	N
	3330 µg	115	N	19	N
	5000 µg	103	N	16	N
Microsomes: None					
Vehicle Control		102	N	12	N
Test Article	6.67 µg	98	N	17	N
	10.0 µg	103	N	14	N
	33.3 µg	94	N	14	N
	66.7 µg	95	N	12	N
	100 µg	80	N	16	N
	333 µg	117	N	18	N
	667 µg	105	N	8	N
	1000 µg	104	N	9	N
	3330 µg	86	R	3	R
	5000 µg	0	R	3	R

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate
Source: Study Report Table 1, (MRID 478990509)

Table 3: Mutagenicity Assay Results – Summary

Test Article ID: MON 52708

Assay No.: 28298-0-409OECD Trial No.: B1

Date Plated: 17-May-06 Vehicle: DMSO

Date Counted: 22-May-06 Plating Aliquot: 50 µL

	Dose/Plate		Mean Revertants Per Plate with Standard Deviation										Background Lawn[c]
			TA98		TA100		TA1535		TA1537		WP2uvrA		
			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver													
Vehicle Control			19	2	85	13	11	3	5	2	16	5	N
Test Article	33.3	µg	18	6	90	12	8	3	7	4	15	3	N
	100	µg	17	7	93	10	10	2	7	1	16	7	N
	333	µg	17	3	94	10	10	4	7	2	15	9	N
	1000	µg	18	5	94	6	9	9	7	2	17	4	N
	2500	µg	17	5	88	10	6	2	6	3	16	1	N
	5000	µg	14	3	97	18	9	4	8	4	8	3	N
Positive Control[b]			261	15	631	106	101	20	71	15	227	47	N
Microsomes: None													
Vehicle Control			13	3	76	2	15	1	5	3	11	4	N
Test Article	33.3	µg	11	2	79	9	13	3	5	3	14	7	N
	100	µg	13	5	73	2	10	4	7	2	12	3	N
	333	µg	9	2	78	0	10	2	6	3	16	2	N
	1000	µg	10	3	72	1	9	4	4	3	16	3	N
	2500	µg	11	3	60	6	6	3	3	2	9	1	N/R[d]
	5000	µg	0	0	8	9	4	5	0	0	7	3	R
Positive Control[c]			285	85	884	53	593	25	225	29	143	36	N

[c] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b]
TA98	benzo[a]pyrene	2.5 µg/plate	[c]	TA98	2-nitrofluorene	1.0 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate		TA100	sodium azide	2.0 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate		TA1535	sodium azide	2.0 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate		TA1537	ICR-191	2.0 µg/plate
WP2uvrA	2-aminoanthracene	25.0 µg/plate		WP2uvrA	4-nitroquinoline-N-oxide	1.0 µg/plate

[d] The first entry is the lawn evaluation for tester strains TA98 and WP2uvrA.
The second entry is the lawn evaluation for tester strains TA100, TA1535, and TA1537.
Source: Study Report, Table 3, p 22 (MRID 478990509).

Table 5: Mutagenicity Assay Results – Summary

Test Article ID: MON 52708

Assay No.: 28298-0-409OECD Trial No.: C1

Date Plated: 02-Jun-06 Vehicle: DMSO

Date Counted: 09-Jun-06 Plating Aliquot: 50 µL

	Dose/Plate	Mean Revertants Per Plate with Standard Deviation										Background Lawn[a]
		TA98		TA100		TA1535		TA1537		WP2uvrA		
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver												
Vehicle Control		20	6	98	15	14	2	10	3	18	3	N
Test Article	33.3 µg	23	3	105	15	11	1	16	4	23	8	N
	100 µg	25	3	105	8	8	2	13	0	23	3	N
	333 µg	23	7	97	22	13	3	12	2	18	7	N
	1000 µg	20	4	94	11	12	4	11	3	17	2	N
	2500 µg	26	3	83	12	14	6	10	2	14	4	N
	5000 µg	25	10	99	3	13	6	10	3	15	3	N
Positive Control[b]		338	38	784	94	129	6	100	31	148	46	N
Microsomes: None												
Vehicle Control		17	2	74	12	12	1	10	3	22	4	N
Test Article	33.3 µg	14	2	79	17	14	1	9	3	20	1	N
	100 µg	15	6	81	23	15	3	13	6	11	1	N
	333 µg	14	5	84	6	12	5	9	2	14	3	N
	1000 µg	13	2	85	11	11	4	8	5	16	5	N
	2500 µg	12	7	74	11	8	5	5	1	12	2	N/R[c]
	5000 µg	1	1	19	8	4	3	3	1	7	3	R
Positive Control[b]		243	24	1261	91	882	14	272	17	235	19	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b] TA98 benzo[a]pyrene 2.5 µg/plate
TA100 2-aminoanthracene 2.5 µg/plate
TA1535 2-aminoanthracene 2.5 µg/plate
TA1537 2-aminoanthracene 2.5 µg/plate
WP2uvrA 2-aminoanthracene 25.0 µg/plate

[c] TA98 2-nitrofluorene 1.0 µg/plate
TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate
TA1537 ICR-191 2.0 µg/plate
WP2uvrA 4-nitroquinoline-N-oxide 1.0 µg/plate

[d] The first entry is the lawn evaluation for tester strains TA100, TA1535, TA1537, and WP2uvrA.
The second entry is the lawn evaluation for tester strain TA98.Source:
Source: Study Report, Table 5, p 24 (MRID 478990509)

Revised by U.S. Environmental Protection Agency

***In vitro* Bacterial Gene Mutation *(Salmonella typhimurium)*/ mammalian activation gene mutation assay**

Report: IIA 5.8/20. Stankowski, L.F., Jr. (2009). Bacterial Reverse Mutation Assay with a Confirmatory Assay. Covance Laboratories, Inc. Vienna, Virginia, USA, Study No. 8202708; unpublished. MRID No. 47899514,

Guidelines: OCSPP 870.5100 (1998)
OECD 471 (1997)
MAFF (Shirasu, 1988)
PRMA DACO 4.5.4

GLP: YES OECD Principles of GLP, ENV/MC/CHEM (1998) 17
Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

GLP Exceptions: No

EXECUTIVE SUMMARY:

In independent trials of a reverse gene mutation assay (MRID No. 47899514), MON 52724 (Purity 96.3%; Lot No.GLP-0903-19699-T), prepared in dimethyl sulfoxide (DMSO) was tested in 4 strains of *S. typhimurium* (TA100, TA98, TA1535 and TA1537) and in *E. coli* WP2*uvr*A at 8 concentrations ranging from 1.6 to 5000 µg/plate with or without S9 activation. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254.

The confirmatory study used a nonactivated concentration range of 3.33 to 2500 ug/plate (*Salmonella* strains) and 10 to 5000 ug/plate (*E. coli*) and an S9-activated test range of 33.3 to 5000 ug/plate (all strains).

Results for both the initial and confirmatory assays were in good agreement and indicate that growth inhibition was observed for all *S. typhimurium* TA strains at ≥1600 ug/plate –S9 and for the *E. coli* strain at 5000 ug/plate –S9; the S9 activated test material was not cytotoxic. The expected responses were achieved with the solvent and positive controls. The mean numbers of revertant colonies for all strains was not appreciably increased by treatment with the test substance at all concentration, with and without S9 activation.

Under the conditions of this study, MON 52724 did not induce gene mutation, either with or without metabolic activation, in any of the *S. typhimurium* TA strains or in the *E. coli* strain up to cytotoxicity and/or the limit dose for this test system.

The study is classified as **totally reliable (acceptable/guideline)** and satisfies the guideline requirements (OCSPP 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: MON 52724, (DCGA)

Description:	White powder
Lot/Batch#:	0903-19699-T
Purity:	96.3%
CAS #:	Not given
Stability of test compound:	Listed with an expiration date of March 12, 2010.
Solvent:	Dimethyl sulfoxide (DMSO)
Structure:	

2. Control Materials

Negative:	None		
Solvent/ concentration	DMSO		
Positive Controls			
Nonactivation	Sodium azide	2.00 ug/plate	TA100,TA1535
	2-nitrofluorene	1.00 µg/plate	TA98
	ICR-191	2.0 ug/plate	TA1537
	4-nitroquinoline-N-oxide	1.0 ug/plate	WP2uvrA
Activated			
	Benzo-(a)pyrene	2.5 µg/plate	TA98
	2-aminoanthracene	2.5 µg/plate	*TA100, TA1535, TA1537*
	2-aminoanthracene	25.0 ug/plate	WP2uvrA

3. Activation: S9 derived from 7 week old, male Sprague-Dawley rats (234 g)

X	Induced	X	Aroclor 1254	X	Rat	X	Liver
	Non-induced		Phenobarbital		Mouse		Lung
			β-naphthoflavone		Hamster		Other (*name*)
			None		Other (*name*)		

The S9 fraction was prepared from a commercially produced liver homogenate from Molecular Toxicology, Inc. (Lot No. 2376 containing 39.8 mg/ml protein). The S9 mix was prepared on the day of use and contained the following components:

S9 Mix Components

Component	Amount
H2O	0.70 mL
1M NaH2PO4/Na2HPO4, pH 7.4	0.10 mL
0.25M Glucose-6-phosphate	0.02 mL
0.10M NADP	0.04 mL
0.825M KCl/0.2M MgCl2	0.04 mL
S9 Homogenate	0.10 mL

The final S9 culture concentration was approximately 10%.

TEST SYSTEM
System Rationale

4. Test organisms:

[ ]	TA97	[X]	TA98	[X]	TA100	[ ]	TA102	[ ]	TA104
[X]	TA1535	[X]	TA1537	[ ]	TA1538	[X]	WP2*uvr*A	[ ]	WP2

Properly maintained?	[X]	Yes	[ ]	No
Checked for appropriate genetic markers (*rfa* mutation, R factor)?	[X]	Yes	[ ]	No

6. Test compound concentrations preparation/used:

MON 52724 and the positive control substances were dissolved in DMSO. The test substance was weighed, dissolved in the requisite volume of solvent to prepare the test solutions at the highest dose level. Test solutions at the lower dose levels were prepared by serial stepwise dilution. Test solutions were prepared at the time of use.

Mutagenicity assay: (Initial)

Non-activated conditions: 0, 1.6, 5.0, 16.0, 50.0, 160, 500, 1600, 5000 µg/plate (all strains)

Activated conditions: 0, 1.6, 5.0, 16.0, 50.0, 160, 500, 1600, 5000 µg/plate (all strains)

(Confirmatory)

Non-activated conditions: 0, 3.33, 10.0, 33.3, 100, 333, 1000, 2500 µg/plate (all Salmonella strains)
0, 10.0, 33.3, 100, 333, 1000, 2500, 5000 µg/plate (E. coli strain)

Activated conditions: 0, 33.3, 100, 333, 1000, 2500, and 5000 µg/plate (all strains)

In both trials of the main assay, triplicate plates were prepared for all test article concentrations, solvent, and positive controls in the presence and absence of S9-activation.

B. STUDY DESIGN AND METHODS

1. In life (experimentation) dates: 24 March 2009 – 21 April 2009.

2. Preliminary cytotoxicity assay: Not performed.

3. Mutation assay: (initial and confirmatory)

For each strain and dose level, 100 µL of tester strain and 50 uL of solvent, test substance solution or positive control solution were mixed with 2.5 ml of molten selective top agar (maintained at 45 ± 2degrees C. After mixing, the material was overlaid on the surface of 25 ml of minimal bottom agar in a 15 X 100 mm petri dish. The dishes were up-ended and incubated at 37°C. After 52±4 hours of incubation, the background lawn was examined visually for precipitation and for signs of growth inhibition. Revertant colonies were counted automatically or manually for the test groups with visible evidence of precipitation and the remaining plates were counted with an automatic colony counter. Viable cell counts were taken for all strains and the sterility of the S9 mix was determined.

4. Statistical analysis:

The data was not subjected to statistical analysis; means and standard deviations were calculated.

5. Evaluation criteria:

The test article was considered to be positive for mutagenicity if the number of revertant colonies of any strain were increased by more that twice the solvent control for strains TA98, TA100, and WP2uvrA, or 3-fold in TA1535 or TA1537, and the response was dose-dependent and reproducible.

NOTE: Historical control data for the solvent controls were presented by the performing laboratory.

II. RESULTS AND DISCUSSION

A. **Initial Mutagenicity assay**: Growth inhibition was seen in the *S. typhimurium* TA strains without metabolic activation at ≥1600 µg/plate and in *E. coli* WP2 *uvrA* at 5000 µg/plate (Table 1). The S9-activated test substance was not cytotoxic for any strain (Table 2). As further shown in Tables 1 and 2, no appreciable increase in mutant colony counts was observed for any strain at any concentration with or without S9. The positive controls induced marked increase in the mutant colony counts for the respective tester strain.

B. **Confirmatory assay**: On the basis of the initial trial data, the highest nonactivated concentration used for the confirmatory mutation assay was set at 2500 µg/plate for the *S. typhimurium* TA strains and 5000 ug/plate the *E. coli* strain. With S9 activation, a maximum dose of 5000 µg/plate used with all strains. Results from the nonactivated and S9-activated confirmatory trial are presented in Tables 3 and 4, respectively. As shown, cytotoxicity was noted for the *S. typhimurium* TA strains at 2500 µg/plate –S9 and clear evidence of cytotoxicity was evident for *E. coli* at 5000 µg/plate –S9. In agreement with

the earlier findings, the S9-activated test material was not cytotoxic. Similarly, the mean numbers of revertant colonies for all strains were not appreciably increased by treatment with the test substance at any concentration, with and without S9. By contrast, the number of revertant colonies for all strain-specific positive controls was clearly increased.

Table 1: Initial Mutagenicity Assay Results with S9

Study No.: 8202708
Trial No.: 8202708-B1 Date Plated: 3/25/2009
Plating Method: Plate incorporation assay Date Counted: 4/1/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	MON 52724	5000	17.5	2.1	0.8	19 N, 16 N
		1600	18.0	1.4	0.8	19 N, 17 N
		500	18.5	4.9	0.9	15 N, 22 N
		160	19.5	12.0	0.9	28 N, 11 N
		50.0	20.0	1.4	0.9	21 N, 19 N
		16.0	14.5	4.9	0.7	18 N, 11 N
		5.00	21.5	0.7	1.0	21 N, 22 N
		1.60	20.0	7.1	0.9	25 N, 15 N
	Dimethyl Sulfoxide		21.5	0.7		22 N, 21 N
TA100	MON 52724	5000	97.5	23.3	1.0	81 N, 114 N
		1600	104.5	2.1	1.1	106 N, 103 N
		500	86.0	5.7	0.9	90 N, 82 N
		160	91.5	6.4	1.0	96 N, 87 N
		50.0	90.5	9.2	1.0	84 N, 97 N
		16.0	90.5	16.3	1.0	102 N, 79 N
		5.00	101.0	15.6	1.1	90 N, 112 N
		1.60	88.5	4.9	1.0	85 N, 92 N
	Dimethyl Sulfoxide		93.0	5.7		89 N, 97 N
TA1535	MON 52724	5000	6.5	0.7	0.4	7 N, 6 N
		1600	9.0	1.4	0.6	8 N, 10 N
		500	7.5	0.7	0.5	7 N, 8 N
		160	6.5	0.7	0.4	7 N, 6 N
		50.0	8.0	2.8	0.5	6 N, 10 N
		16.0	12.0	1.4	0.8	11 N, 13 N
		5.00	7.0	1.4	0.5	8 N, 6 N
		1.60	6.0	1.4	0.4	5 N, 7 N
	Dimethyl Sulfoxide		15.0	0.0		15 N, 15 N
TA1537	MON 52724	5000	2.5	2.1	0.4	1 N, 4 N
		1600	8.0	2.8	1.2	6 N, 10 N
		500	8.0	0.0	1.2	8 N, 8 N
		160	5.0	1.4	0.8	4 N, 6 N
		50.0	4.5	3.5	0.7	7 N, 2 N
		16.0	6.5	2.1	1.0	5 N, 8 N
		5.00	7.5	0.7	1.2	7 N, 8 N
		1.60	4.0	0.0	0.6	4 N, 4 N
	Dimethyl Sulfoxide		6.5	0.7		6 N, 7 N
WP2*uvrA*	MON 52724	5000	13.0	4.2	0.8	10 N, 16 N
		1600	12.0	1.4	0.8	13 N, 11 N
		500	10.5	0.7	0.7	10 N, 11 N
		160	13.0	4.2	0.9	18 M B N, 12 N
		50.0	20.0	2.8	1.3	18 N, 22 N
		16.0	16.0	0.0	1.0	16 N, 16 N
		5.00	21.0	2.8	1.3	19 N, 23 N
		1.60	16.5	6.4	1.0	12 N, 21 N
	Dimethyl Sulfoxide		16.0	4.2		13 N, 19 N
TA98	BP	2.5	403.0	26.9	18.7	422 N, 384 N
TA100	2AA	2.5	1408.5	27.6	15.1	1389 N, 1428 N
TA1535	2AA	2.5	186.5	9.2	12.4	180 N, 193 N
TA1537	2AA	2.5	88.0	22.6	13.5	104 N, 72 N
WP2*uvrA*	2AA	25.0	386.5	6.4	24.2	391 N, 382 N

Key to Positive Controls

BP Benzo{a}pyrene

2AA 2-aminoanthracene

Key to Plate Postfix Codes

N Normal background bacterial lawn

M Plate counted manually

B Bubbles or Split in agar

Source: Study Report Table 1, pp 22-23 (MRID 47899514).

Table 2: Initial Mutagenicity Assay Results without S9

Study No.: 8202708
Trial No.: 8202708-B1 Date Plated: 3/25/2009
Plating Method: Plate incorporation assay Date Counted: 4/1/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	MON 52724	5000	0.0	0.0	0.0	0 A, 0 A
		1600	0.0	0.0	0.0	0 A, 0 A
		500	16.5	0.7	1.1	16 N, 17 N
		160	18.0	4.2	1.2	21 N, 15 N
		50.0	13.0	0.0	0.9	13 N, 13 N
		16.0	10.5	0.7	0.7	11 N, 10 N
		5.00	15.5	3.5	1.0	18 N, 13 N
		1.60	13.0	2.8	0.9	11 N, 15 N
	Dimethyl Sulfoxide		15.0	4.2		12 N, 18 N
TA100	MON 52724	5000	0.0	0.0	0.0	0 A, 0 A
		1600	0.0	0.0	0.0	0 A, 0 A
		500	87.5	21.9	1.0	103 N, 72 N
		160	103.0	28.3	1.2	125 N, 85 N
		50.0	72.0	14.1	0.8	82 N, 62 N
		16.0	98.0	7.1	1.1	93 N, 103 N
		5.00	82.5	6.4	0.9	87 N, 78 N
		1.60	88.5	3.5	1.0	91 N, 86 N
	Dimethyl Sulfoxide		91.0	1.4		90 N, 92 N
TA1535	MON 52724	5000	0.0	0.0	0.0	0 A, 0 A
		1600	0.0	0.0	0.0	0 A, 0 A
		500	9.0	4.2	0.7	12 N, 6 N
		160	7.0	0.0	0.6	7 N, 7 N
		50.0	8.0	4.2	0.6	5 N, 11 N
		16.0	17.5	0.7	1.4	18 M B N, 17 N
		5.00	15.5	10.6	1.2	23 N, 8 N
		1.60	11.5	0.7	0.9	12 N, 11 N
	Dimethyl Sulfoxide		12.5	0.7		13 N, 12 N
TA1537	MON 52724	5000	0.0	0.0	0.0	0 A, 0 A
		1600	0.0	0.0	0.0	0 A, 0 A
		500	8.0	3.7	1.2	12 N, 4 N
		160	6.0	3.7	0.9	10 N, 2 N
		50.0	6.5	2.1	1.0	8 N, 5 N
		16.0	5.0	1.4	0.8	6 N, 4 N
		5.00	3.0	2.8	0.5	1 N, 5 N
		1.60	5.5	0.7	0.8	6 N, 5 N
	Dimethyl Sulfoxide		6.5	2.1		5 N, 8 N
WP2uvrA	MON 52724	5000	0.0	0.0	0.0	0 A, 0 A
		1600	14.5	3.5	0.8	12 N, 17 N
		500	22.5	9.2	1.3	16 N, 29 N
		160	17.5	8.2	1.0	11 N, 24 N
		50.0	15.0	2.8	0.9	17 N, 13 N
		16.0	17.0	2.8	1.0	15 N, 19 N
		5.00	14.5	2.1	0.8	16 N, 13 N
		1.60	17.5	2.1	1.0	19 N, 16 N
	Dimethyl Sulfoxide		17.5	6.4		22 N, 13 N
TA98	2NF	1.0	295.5	14.8	19.7	285 N, 306 N
TA100	SA	2.0	1140.5	17.7	12.5	1153 N, 1128 N
TA1535	SA	2.0	794.5	14.8	63.6	784 N, 805 N
TA1537	ICR	2.0	288.0	69.3	44.3	337 N, 239 N
WP2uvrA	4NQO	1.0	184.5	19.1	10.3	171 N, 198 N

Key to Positive Controls

2NF 2-nitrofluorene
SA sodium azide
ICR ICR-191
4NQO 4-nitroquinoline-N-oxide

Key to Plate Postfix Codes

A Absence of background bacterial lawn
N Normal background bacterial lawn
M Plate counted manually
B Bubbles or Split in agar

Source: Study Report Table 2, pp 24-25 (MRID 47899514).

Table 3: Confirmatory Mutagenicity Assay Results with S9

Study No.: 8202708
Trial No.: 8202708-C1 Date Plated: 4/10/2009
Plating Method: Plate incorporation assay Date Counted: 4/16/2009 to 4/21/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	**MON 52724**	5000	*12.7*	*3.8*	*0.7*	17 N, 10 N, 11 N
		2500	*14.7*	*4.5*	*0.8*	15 N, 10 N, 19 N
		1000	*19.3*	*3.1*	*1.1*	20 N, 22 N, 16 N
		333	*30.0*	*5.7*	*1.7*	C, 26 N, 34 N
		100	*17.3*	*1.5*	*1.0*	19 N, 17 N, 16 N
		33.3	*30.7*	*9.8*	*1.8*	25 N, 42 N, 25 N
	Dimethyl Sulfoxide		*17.3*	*2.3*		20 N, 16 N, 16 N
TA100	**MON 52724**	5000	*129.0*	*7.9*	*1.1*	138 N, 126 N, 123 N
		2500	*113.0*	*6.9*	*1.0*	111 N, 123 N, 111 N
		1000	*108.3*	*9.0*	*0.9*	117 N, 99 N, 109 N
		333	*119.0*	*6.2*	*1.0*	121 N, 124 N, 112 N
		100	*118.0*	*13.2*	*1.0*	123 N, 103 N, 128 N
		33.3	*118.0*	*9.5*	*1.0*	107 N, 124 N, 123 N
	Dimethyl Sulfoxide		*115.7*	*6.5*		116 N, 109 N, 122 N
TA1535	**MON 52724**	5000	*9.7*	*2.5*	*0.7*	10 N, 12 N, 7 N
		2500	*13.7*	*3.2*	*1.0*	16 N, 15 N, 10 N
		1000	*10.0*	*3.6*	*0.7*	9 N, 14 N, 7 N
		333	*10.7*	*5.1*	*0.7*	20 N, 7 N, 5 N
		100	*13.3*	*5.1*	*0.9*	19 N, 9 N, 12 N
		33.3	*11.7*	*2.5*	*0.8*	9 N, 14 N, 12 N
	Dimethyl Sulfoxide		*14.3*	*4.0*		12 N, 19 N, 12 N
TA1537	**MON 52724**	5000	*3.0*	*1.0*	*0.5*	6 N, 4 N, 5 N
		2500	*5.7*	*3.5*	*0.6*	9 N, 6 N, 2 N
		1000	*8.3*	*3.8*	*0.9*	11 N, 10 N, 4 N
		333	*6.3*	*0.6*	*0.7*	6 N, 7 N, 6 N
		100	*6.3*	*4.0*	*0.7*	11 N, 4 N, 4 N
		33.3	*6.0*	*3.6*	*0.6*	2 N, 7 N, 9 N
	Dimethyl Sulfoxide		*9.3*	*2.5*		9 N, 7 N, 12 N
WP2*uvrA*	**MON 52724**	5000	*16.7*	*4.9*	*1.1*	19 N, 11 N, 20 N
		2500	*12.7*	*6.4*	*0.9*	9 N, 9 N, 20 N
		1000	*18.7*	*7.1*	*1.3*	11 N, 20 N, 25 N
		333	*22.0*	*3.6*	*1.5*	21 N, 26 N, 19 N
		100	*15.0*	*0.0*	*1.0*	15 N, 15 N, 15 N
		33.3	*16.0*	*3.5*	*1.1*	14 N, 14 N, 20 N
	Dimethyl Sulfoxide		*14.7*	*5.0*		10 N, 14 N, 20 N
TA98	**BP**	2.5	*241.3*	*12.5*	*13.9*	229 N, 254 N, 241 N
TA100	**2AA**	2.5	*1298.3*	*155.8*	*11.2*	1121 N, 1413 N, 1361 N
TA1535	**2AA**	2.5	*257.3*	*11.2*	*18.0*	267 N, 260 N, 245 N
TA1537	**2AA**	2.5	*106.7*	*7.5*	*11.4*	111 N, 98 N, 111 N
WP2*uvrA*	**2AA**	25.0	*591.3*	*101.4*	*40.3*	661 N, 638 N, 475 N

Key to Positive Controls

BPBenzo{a}pyrene

2AA 2-aminoanthracene

Key to Plate Postfix Codes

N Normal background bacterial lawn

M Plate counted manually

Source: Study Report Table 3, pp 26-27 (MRID 47899514).

Table 4: Confirmatory Mutagenicity Assay Results without S9

Study No.: 8202708
Trial No.: 8202708-C1 Date Plated: 4/10/2009
Plating Method: Plate incorporation assay Date Counted: 4/16/2009 to 4/21/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	MON 52724	2500	1.3	1.5	0.1	1 R, 3 R, 0 R
		1000	16.3	7.5	1.0	25 N, 12 N, 12 N
		333	9.3	2.1	0.6	11 N, 7 N, 10 N
		100	16.0	7.2	1.0	14 N, 24 N, 10 N
		33.3	13.7	6.5	0.9	14 N, 7 N, 20 N
		10.0	13.3	2.1	0.9	14 N, 15 N, 11 N
		3.33	19.7	0.6	1.3	20 N, 20 N, 19 N
	Dimethyl Sulfoxide		15.7	4.0		15 N, 12 N, 20 N
TA100	MON 52724	2500	26.7	44.5	0.2	2 R, 78 R, 0 R
		1000	104.3	2.5	0.9	103 R, 107 R, 103 R
		333	104.3	13.1	0.9	92 N, 118 N, 103 N
		100	86.3	9.1	0.8	96 N, 78 N, 85 N
		33.3	91.0	12.2	0.8	97 N, 99 N, 77 N
		10.0	93.0	28.6	0.8	78 N, 126 N, 75 N
		3.33	100.0	12.1	0.9	89 N, 113 N, 98 N
	Dimethyl Sulfoxide		114.7	11.5		108 N, 128 N, 108 N
TA1535	MON 52724	2500	5.3	4.7	0.3	9 R, 7 R, 0 R
		1000	11.0	0.0	0.6	11 N, 11 N, 11 N
		333	11.7	2.1	0.6	10 N, 14 N, 11 N
		100	10.7	1.2	0.5	12 N, 10 N, 10 N
		33.3	14.3	2.5	0.7	17 N, 14 N, 12 N
		10.0	12.7	2.9	0.6	16 N, 11 N, 11 N
		3.33	17.0	3.0	0.9	14 N, 17 N, 20 N
	Dimethyl Sulfoxide		19.7	4.0		22 N, 22 N, 15 N
TA1537	MON 52724	2500	1.7	0.6	0.6	2 R, 2 R, 1 R
		1000	5.0	1.0	1.9	6 R, 4 R, 5 R
		333	7.3	3.2	2.8	6 N, 11 N, 5 N
		100	6.7	2.1	2.5	5 N, 6 N, 9 N
		33.3	11.3	2.5	4.3	14 N, 11 N, 9 N
		10.0	8.7	4.0	3.3	11 N, 4 N, 11 N
		3.33	5.7	5.0	2.1	1 N, 11 N, 5 N
	Dimethyl Sulfoxide		2.7	2.9		1 N, 1 N, 6 N
WP2uvrA	MON 52724	5000	8.0	1.7	0.3	7 R, 7 R, 10 R
		2500	14.0	3.5	0.8	16 R, 16 R, 10 R
		1000	20.0	2.6	1.1	21 R, 22 R, 17 R
		333	20.3	4.7	1.2	22 N, 15 N, 24 N
		100	12.3	1.5	0.7	11 N, 14 N, 12 N
		33.3	17.3	3.5	1.0	21 N, 17 N, 14 N
		10.0	17.3	1.5	1.0	17 N, 16 N, 19 N
	Dimethyl Sulfoxide		17.7	5.3		19 N, 12 N, 22 N
TA98	2NF	1.0	426.7	32.7	27.2	447 N, 444 N, 389 N
TA100	SA	2.0	1354.9	68.5	11.8	1344 N, 1291 N, 1427 N
TA1535	SA	2.0	984.0	47.3	30.0	999 N, 1022 N, 931 N
TA1537	ICR	2.0	159.3	18.2	39.8	165 N, 139 N, 174 N
WP2uvrA	4NQO	1.0	191.3	27.2	10.8	209 N, 205 N, 160 N

Key to Positive Controls

2NF 2-nitrofluorene
SA sodium azide
ICR ICR-191
4NQO 4-nitroquinoline-N-oxide

Key to Plate Postfix Codes

R Reduced background bacterial lawn
N Normal background bacterial lawn

Source: Study Report Table 4, pp 28-29 (MRID 47899514).

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:

Reliability rating: this study is **totally reliable and acceptable**.

This study is compliant with OECD 471(1997) with the exception that the test doses were not analyzed at the moment of use.

C. CONCLUSIONS:

MON 52724 was tested up to cytotoxic concentrations (≥1600 µg/plate-S9) in all Salmonella strains and at 5000 µg/plate-S9 in the *E. coli* strain but was not cytotoxic in the presence of S9 activation. Similarly, the test material was not mutagenic in any strain either in the presence or the absence of S9 activation at any concentration. The respective positive controls induced marked increases in revertant colonies in the appropriate strain in the presence or absence of S9-activation.

Accordingly, MON 52724 is considered to be negative for mutagenicity in this test system in a reasonably well-done study.

Deficiencies: None

Revised by U.S. Environmental Protection Agency

In vitro Bacterial Gene Mutation (Salmonella typhimurium)/ mammalian activation gene mutation assay

Report: IIA 5.8/20. Mecchi, M. (2006). Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay with a confirmatory assay with MON 11900; Covance Laboratories Inc. Vienna, Virginia, Monsanto Study CV-2006-081, 29 September 2006, unpublished. MRID 47899525.

Guidelines: OPPTS 870.5100 (1998)
OECD 471 (1997)
JMAFF (Shirasu, 1988)

GLP: YES OECD Principles of GLP, ENV/MC/CHEM (1998) 17
Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

GLP
Exceptions: None

EXECUTIVE SUMMARY:

In independent trials of a reverse gene mutation assay (MRID No47899525), MON 11900 (Purity 94.6%; Lot No. GLP-0604-17184-T) prepared in dimethyl sulfoxide (DMSO) was tested in 4 strains of S. typhimurium (TA100, TA98, TA1535 and TA1537) and in E. coli WP2uvrA at 6 concentrations ranging from 33.3 to 5000 µg/plate with or without S9 activation. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254.

Levels for the main assay were determined from the results of a dose range-finding study in S. typhimurium TA100 strain and the E. coli 1 strain in which concentrations of 6.67 - 5000 µg/plate and the relevant solvent and positive controls, both with and without metabolic activation were assayed. Following incubation for 48 hours, growth inhibition due to the nonactivated test substance was observed at $\geq$ 3300 µg/plate (S. typhimurium TA100) and at 5000 µg/plate (E. coli). The S9-activated test material was not cytotoxic at any level. Accordingly, the highest level used for the main assays was set at 5000 µg/plate +/-S9.

Reductions in the background lawn of growth and/or the mutant colony counts was observed in all strain at 5000 µg/plate –S9; the S9-activated test substance was not cytotoxic. However, the mean numbers of revertant colonies for all strains were not appreciably increased by treatment with the test substance at all concentrations dose levels, with or without S9. The numbers of revertant colonies in all strain-specific positive control groups were clearly increased and were within the laboratory historical control ranges.

Under the conditions of this study, MON 11900 did not induce gene mutation, either with or without metabolic activation, in any of the S. typhimurium TA strains up to concentrations that inhibited background lawn cell growth or in the E. coli strain up to the limit dose for this test system (5000 µg/plate).

The study is classified as **totally reliable (acceptable/guideline)** and satisfies the guideline requirements (OCSPP 870.5100; OECD 471) for in vitro mutagenicity (bacterial reverse gene mutation) data.

IIIA 9 I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	MON 11900 (Purity 94.6%; Lot No. GLP-0604-17184-T
Description:	White solid
Lot/Batch#:	GLP-0604-17184-T
Purity:	94.6%
CAS #:	Not given
Stability of test compound:	Listed with an expiration date of April 26, 2007
Solvent:	Dimethyl sulfoxide (DMSO)

2. Control Materials

Negative:	None		
Solvent/concentration	DMSO/0.05mL		
Positive			
Nonactivation	2-nitrofluorene	1.0µg/plate	TA100, E.coli WP2uvrA
	ICR-191	2.0 ug/plate	TA1537
	Sodium azide; NaN_3	2.0 µg/plate	TA100, TA1535
	4-nitroquinoline-N-oxide	1.0 µg/plate	WP2uvrA
Activated	Benzo(a)pyrene; B(a)P	2.5. µg/plate	TA98
	2-Aminoanthracene: 2-AA	2.5 µg/plate	TA1535,TA100, TA1537
		25.0 µg/plate	E. coli WP2uvrA

3. Activation: S9 derived from male Sprague-Dawley rats

X	Induced	X	Aroclor 1254	X	Rat	X	Liver
	Non-induced		Phenobarbital		Mouse		Lung
			β-naphthoflavone		Hamster		Other (name)
			None		Other (name)		

The S9 fraction was prepared commercially by Molecular Toxicology, Inc. (Lot No. 2001; 40.5 mg protein/mL). The S9 fraction (1 ml) was added to a cofactor mix, which contained the following components:

S9 Mix Components

Component	Amount

H2O	0.70 mL
1M NaH2PO4/Na2HPO4, pH 7.4	0.10 mL
0.25M Glucose-6-phosphate	0.02 mL
0.10M NADP	0.04 mL
0.825M KCl/0.2M MgCl2	0.04 mL
S9 Homogenate	0.10 mL

The final S9 culture concentration was approximately 10%.

4. Test organisms:

[ ]	TA97	[X]	TA98	[X]	TA100	[ ]	TA102	[ ]	TA104
[X]	TA1535	[X]	TA1537	[ ]	TA1538	[X]	WP2uvrA	[ ]	WP2

Properly maintained?	[X]	Yes	[ ]	No
Checked for appropriate genetic markers (rfa mutation, R factor)?	[X]	Yes	[ ]	No

7. Test compound concentrations preparation/used:

MON 11900 and the positive control substances were dissolved in DMSO. The test substance was weighed, dissolved in the requisite volume of solvent to prepare the test solutions at the highest dose level. Test solutions at the lower dose levels were prepared by serial stepwise dilution. Test solutions were prepared at the time of use.

Dose range-finding cytotoxicity assay: Ten doses ranging from 6.67 to 5000 ug/plate were tested (S. typhimurium TA100 and the E. coli strain, +/- S9).

Mutagenicity assay:

Non-activated conditions: 0, 33.3, 100, 333, 1000, 2500 and 5000 µg/plate (both trials)

Activated conditions: 0, 33.3, 100, 333, 1000, 2500, 5000 µg/plate (both trials)

In both trials of the main assay, triplicate plates were prepared for all test article concentrations, solvent, and positive controls in the presence and absence of S9-activation.

B. STUDY DESIGN AND METHODS

1. In life (experimentation) dates: June 15, 2006 – July 20, 2006

2. Preliminary cytotoxicity assay:

Levels for the main assay were determined from the results of the preliminary cytotoxicity test, which was performed as described below for the plate incorporation mutation assay.

3. Mutation assay:

When S9 mix was not required, 100 µL of tester strain and 50 µL of vehicle, positive control or test article dose were added to 2.5 mL of molten selective top agar (maintained at 45 ± 2°C). When S9 mix was required, 500 µL of S9 mix, 100 µL of tester strain and 50 µL of vehicle, positive control or test article dose were added to 2.0 mL of molten selective top agar. The mixture was vortexed and overlaid onto the surface of 25 mL of minimal bottom agar contained in a 15 x 100 mm petri dish. After the overlay solidified, the plates were inverted and incubated for 52 ± 4 hours at 37 ± 2°C.

4. Statistical analysis:

The data was not subjected to statistical analysis; but means and standard deviations were calculated.

5. Evaluation criteria:

The test article was considered to be positive for mutagenicity if the number of revertant colonies of any strain were increased by more that twice the solvent control, and the response was dose-dependent and reproducible for strains TA98, TA100, and WP2uvrA or at least a 3-fold increase in the mean revertants per plate of TA1535 or TA1537.

NOTE: The performing laboratory presented historical control data for the solvent and positive controls.

IIIA 10 II. RESULTS AND DISCUSSION

A. Preliminary cytotoxicity assay: Compound precipitation was not observed at any concentration. In the absence of S9 activation, cytotoxicity, as indicated by a reduction in the bacterial lawn of growth and/or in mutant colonies was seen for both TA100 and E. coli at 5000 µg /plate. With S9, the test material was not cytotoxic. Based on these results, 5000 µg/plate was selected as the starting level for the mutation assays.

B. Mutation assay: Summarized results form both trials are presented in Tables 3 and 5 of the Study Report. As shown, reductions in the background lawn of growth were noted for all Salmonella strains at 5000 µg/plate –S9 but not in the E. coli strain. In the presence of S9 activation, the test article was not cytotoxic. Similarly, the mean numbers of revertant colonies for all strains were not appreciably increased by treatment with the test substance at all concentrations dose levels, with and without S9. These findings were confirmed in Trial 2. By contrast, the numbers of revertant colonies in all strain-specific positive control groups were clearly increased in both trials and the numbers of revertant colonies in the solvent and all positive control groups were within the laboratory historical control range.

IIIA 10.1.1 III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:

RELIABILITY RATING: Totally reliable. This study is compliant with OECD 471(1997)

C. CONCLUSIONS:
MON 11900 was tested up to the limit dose (5000 µg/plate), which was cytotoxic in the absence of S9 activation for the Salmonella strains but not the E. coli strain and failed to induce increases in the mean number of revertants/plate in any strain (+/-S9). The positive controls induced marked increases in revertant colonies compared to controls in all strains in the presence and absence of S9-activation.

Accordingly, MON11900 is negative in this test system in a well-done study.

Table 1: Dose Rangefinding Study

Test Article ID: MON 11900

Assay No.: 28354-0-409OECD Trial No.: A1

Date Plated: 15-Jun-06 Vehicle: DMSO

Date Counted: 20-Jun-06 Plating Aliquot: 50 µL

			Revertants per Plate			
	Dose/Plate		TA100	Background Lawn[a]	WP2uvrA	Background Lawn[a]
Microsomes: Rat Liver						
Vehicle Control			106	N	20	N
Test Article	6.67	µg	95	N	28	N
	10.0	µg	104	N	12	N
	33.3	µg	111	N	14	N
	66.7	µg	81	N	18	N
	100	µg	94	N	19	N
	333	µg	96	N	17	N
	667	µg	106	N	25	N
	1000	µg	91	N	20	N
	3330	µg	98	N	24	N
	5000	µg	89	N	12	N
Microsomes: None						
Vehicle Control			78	N	10	N
Test Article	6.67	µg	76	N	7	N
	10.0	µg	79	N	14	N
	33.3	µg	88	N	21	N
	66.7	µg	81	N	11	N
	100	µg	88	N	8	N
	333	µg	93	N	19	N
	667	µg	85	N	16	N
	1000	µg	79	N	7	N
	3330	µg	65	R	16	N
	5000	µg	51	R	16	R

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

Source: Study Report Table 1, p 21 (MRID 47899525)

Table 3: Mutagenicity Assay Results Summary

Test Article ID: MON 11900

Assay No.: 28354-0-409OECD Trial No.: B1

Date Plated: 27-Jun-06 Vehicle: DMSO

Date Counted: 30-Jun-06 Plating Aliquot: 50 µL

			Mean Revertants Per Plate with Standard Deviation										Back-ground Lawn[a]
	Dose/Plate		TA98		TA100		TA1535		TA1537		WP2uvrA		
			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver													
Vehicle Control			17	7	102	23	12	3	8	1	17	2	N
Test Article	33.3	µg	22	9	101	8	11	3	5	3	19	8	N
	100	µg	18	7	100	9	12	4	8	2	18	2	N
	333	µg	20	3	112	8	11	2	7	2	16	9	N
	1000	µg	20	8	95	6	12	4	11	6	17	6	N
	2500	µg	21	4	113	7	13	5	10	5	16	8	N
	5000	µg	16	7	100	5	8	2	9	2	14	7	N
Positive Control[b]			290	40	562	63	112	11	99	2	290	38	N
Microsomes: None													
Vehicle Control			12	5	94	4	13	3	3	1	13	3	N
Test Article	33.3	µg	15	1	86	17	12	6	7	2	12	3	N
	100	µg	16	5	94	1	10	2	5	3	16	3	N
	333	µg	15	3	92	7	13	5	6	2	18	2	N
	1000	µg	13	3	88	17	11	6	8	3	15	7	N
	2500	µg	8	3	88	3	9	2	6	3	15	6	N
	5000	µg	4	3	67	4	6	4	3	1	17	1	N/R[d]
Positive Control[c]			213	39	1185	23	901	59	240	67	240	37	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b] TA98 benzo[a]pyrene 2.5 µg/plate
TA100 2-aminoanthracene 2.5 µg/plate
TA1535 2-aminoanthracene 2.5 µg/plate
TA1537 2-aminoanthracene 2.5 µg/plate
WP2uvrA 2-aminoanthracene 25.0 µg/plate

[c] TA98 2-nitrofluorene 1.0 µg/plate
TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate
TA1537 ICR-191 2.0 µg/plate
WP2uvrA 4-nitroquinoline-N-oxide 1.0 µg/plate

[d] The first entry is the lawn evaluation for tester strain WP2uvrA.
The second entry is the lawn evaluation for tester strains TA98, TA100, TA1535, and TA1537.

Source: Study Report Table 3, p 22 (MRID 47899525)

Table 5: Mutagenicity Assay Results - Summary

Test Article ID: MON 11900

Assay No.: 28354-0-409OECD Trial No.: C1

Date Plated: 14-Jul-06 Vehicle: DMSO

Date Counted: 20-Jul-06 Plating Aliquot: 50 µL

	Dose/Plate		Mean Revertants Per Plate with Standard Deviation										Background Lawn[a]
			TA98		TA100		TA1535		TA1537		WP2uvrA		
			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver													
Vehicle Control			20	4	89	12	9	1	5	3	16	2	N
Test Article	33.3	µg	20	8	91	14	7	2	4	2	17	1	N
	100	µg	20	3	79	12	11	5	6	2	17	3	N
	333	µg	15	4	88	5	10	4	8	1	14	5	N
	1000	µg	17	1	102	6	9	5	6	1	17	4	N
	2500	µg	19	3	92	6	8	1	2	1	15	2	N
	5000	µg	18	2	98	14	9	3	6	2	13	4	N
Positive Control[b]			366	39	1120	91	109	22	123	10	347	13	N
Microsomes: None													
Vehicle Control			12	6	82	7	10	4	5	3	12	3	N
Test Article	33.3	µg	12	1	81	8	9	2	7	2	9	1	N
	100	µg	16	2	81	9	7	4	7	3	18	3	N
	333	µg	8	7	84	8	10	2	6	3	19	6	N
	1000	µg	10	3	82	10	8	3	4	3	17	2	N
	2500	µg	9	6	70	9	10	4	4	2	16	7	N
	5000	µg	2	3	37	15	4	4	1	1	14	4	NR[d]
Positive Control[b]			241	23	980	13	693	44	317	57	170	28	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b] TA98	benzo[a]pyrene	2.5 µg/plate	[c] TA98	2-nitrofluorene	1.0 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate	TA100	sodium azide	2.0 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate	TA1535	sodium azide	2.0 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate	TA1537	ICR-191	2.0 µg/plate
WP2uvrA	2-aminoanthracene	25.0 µg/plate	WP2uvrA	4-nitroquinoline-N-oxide	1.0 µg/plate

[d] The first entry is the lawn evaluation for tester strain WP2uvrA.
The second entry is the lawn evaluation for tester strains TA98, TA100, TA1535, and TA1537.

Source: Study Report Table 5, p 25 (MRID 47899525)

IIIA 10.1.1.1 Revised by U.S. Environmental Protection Agency

In Vitro Gene Mutation assay in Chinese hamster cells HGPRT

Report: IIA 5.4.2/01. Cifone, M., (2006). CHO HGPRT forward mutation assay with a confirmatory assay and duplicate cultures with MON 52708. Covance Laboratories Inc., Vienna, Virginia 22182-1699 Monsanto Study No. CV-2006-055, September 29, 2006. unpublished.. MRID No. 47899512

Dates of work: April 27, 2006- June 23, 2006.

Guidelines: OCSPP 870. 5300 (August, 1998)
OECD 476 (July 21, 1997)
JMAFF –(Shirsu-1988)

GLP: Yes USEPA Principles of GLP, ENV/MC/CHEM (1989)
Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

GLP: Exceptions No

EXECUTIVE SUMMARY:

In independently performed mammalian cell forward gene mutation assays (MRID 47899512), Chinese hamster ovary cells at the HGPRT locus were exposed for 4 hours to MON 52708 (Purity 97.9%; Lot/Batch No. GLP-0603-16958-T), prepared in dimethyl sulfoxide (DMSO), at concentrations of 0, 200, 400, 600, 800, 1000, 1200, and 1600 µg/mL -S9 and 0, 200, 400, 500, 600,700, 800, 1000, and 1200 µg/mL +S9 (Initial trial). For the confirmatory trial levels of 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, and 1600 µg/mL –S9 and 0, 500, 600, 700, 800, 900, 1000, and 1200 µg/mL +S9 were assayed. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Arclor 1254.

Test levels for the main assay were determined following a preliminary cytotoxicity test with concentrations ranging from 4.93 to 2500 µg/mL +/-S9. Based on the finding of total lethality at 2500 µg/mL-S9, accompanied by a clonal efficiency (CE) rate of 17% at 1250 µg/mL-S9, and total lethality at ≥1250 µg/mL+S9 the high levels selected for the initial trial were 1600 µg/mL-S9 and 1200 µg/mL +S9.

Concentrations of 1600 µg/mL-S9 and 1200 µg/mL +S9 were severely cytotoxic and discarded. At the highest plated nonactivated concentrations, percent relative population growth (RPG) was ≤10% (1400 µg/mL, initial trial) or ≤28% (1600 µg/mL, confirmatory trial). With S9, (RPG) was ≤15% (1000 µg/mL, initial trial) or ≤8% (1200 µg/mL, confirmatory trial). The positive controls induced the expected mutagenic responses in both trials. In the initial trial, isolated significant increases in the mutation frequency (MF) were noted at 400 µg/mL-S9 (initial trial) and at 400, 500, 600 and 1000 µg/mL+S9. These increases were neither dose-related nor replicate in the duplicate culture. No significant increases in the MF were seen in the confirmatory nonactivated trial. With S9, isolated and significant increases in the MF were recorded for single cultures at 700, 800, 1000, and 1100 µg/mL. These responses were not seen at comparable doses in the initial trial, occurred in only 1 of 2 replicates and generally did not exceed the MF (15×10^{-6}) required to compensate for random fluctuations in the MF.

Based on these considerations, it was concluded that under the conditions of this study, MON 52708 did not induce forward mutations at the HGPRT locus in CHO cells with and without metabolic activation.

This study is classified as reasonably **totally reliable (acceptable/guideline)** and satisfies the guideline requirement for In vitro CHO HGPRT Forward mutation Assay of OCSPP 870.5300; OECD 476.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	MON 52708 (DCSA)
Description:	White powder
Lot/Batch#:	GLP-0603-16958-T
Purity:	97.9%
CAS #:	Not given
Stability of test compound:	Stability information not performed for assay

2. Control materials

Negative control:	None
Solvent control:	DMSO /1% (10 uL/mL)
Positive control:	Non-activation: 5-Bromo-2"-deoxyuridine (BrdU at 50 µg/mL)
	Activation: 3-Methylcholanthrene (MCA at 5 µg/mL)

3. Activation: S9 derived from male Sprague-Dawley rats

X	Induced	X	Aroclor 1254	X	Rat	X	Liver
	Non-induced		Phenobarbital		Mouse		Lung
			β-naphthoflavone		Hamster		Other
			Other		Other		

The S9 fraction was prepared commercially and the S9 mix contained the following components:

IIIA 10.1.2 S9 Mix Components

Component	Amount
NADP (sodium salt)	0.8 mM
Glucose-6-phosphate	1.0 mM
Calcium chloride	2.0 mM
Potassium chloride	6.0 mM
Magnesium Chloride	2.0 mM
Phosphate	10.0 mM
S9 homogenate	10.0 uL/mL

4. Test cells: Mammalian cells in culture

	Mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
X	Chinese hamster ovary (CHO) cells		list any others
	Human peripheral lymphocytes		

Properly maintained?	X	Yes		No
Periodically checked for Mycoplasma contamination?	X	Yes		No
Periodically checked for karyotype stability?	X	Yes		No

Media: Ham' Nutrient Mixture supplemented with L-glutamine , gentamicin, Fungizone, and 8% v/v fetal bovine serum .

5. Locus examined

Selection agent:

	Thymidine kinase (TK)	X	Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)		Na^+/K^+ ATPase
	Bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		Ouabain
	Fluorodeoxyuridine (FdU)	X	6-thioguanine (6-TG; 24μ M)		
X	Trifluorothymidine (TFT)				

6. Test compound concentrations used:

a. Preliminary cytotoxicity (triplicate plates from single culture for each test group)

Concentrations tested (μg/mL)

4 hours, without S9	0	78.5*	157	313	625	1250	2500
4 hours, with S9	0	78.5*	157	313	625	1250	2500

- Lower concentrations down to 4.93 μg/mL+/-S9 were tested.

b. Mutagenicity assay (triplicate plates for survival & 12 plates for mutant selection from duplicate cultures per test group)—both trials

Concentrations tested (μg/mL)

4 hours, without S9 Initial trial	0	200			400	600	800	1000	1200	1400	1600
4 hours, without S9 Conf. Trial	0		800	900	1000	1100	1200	1300	1400	1500	1600
4 hours, with S9 Initial trial	0	200			400	500	600	700	800	1000	1200
4 hours, with S9 Conf. Trial	0		500	600	700	800	900	1000	1100	1200	

IIIA 10.1.3 B. STUDY DESIGN AND METHODS

1. In life dates: February 24 2000 – August 1 2000.

2. Treatment:

Preliminary cytotoxicity assay: Concentrations for the main study were determined from the results of a preliminary cell growth inhibition test in which dishes of 200 cells each (3 dishes/concentration) were exposed to a series of 10 levels of the test material for 4 hours, with and without S9, in a 4-6% CO2 atmosphere. The cells were washed with Dulbecco's phosphate buffered saline and then incubated in F12 culture medium for 7 days. The dishes were washed and the colonies were fixed in methanol and stained with Giemsa and counted manually. Cytotoxicity was expressed as a percentage of mean cloning efficiency (CE) for each level as compared to the vehicle controls.

Mutation assay: As stated in the study report:

Nonactivation Assay

"The cleansed cells were plated at 4 x 106 cells per T-75 (75 cm2) tissue culture flask on the day before dosing. At the time of dosing, cell cultures were treated with the test or control article for about 4 hours at 35-38°C in a humidified atmosphere with 4-6% CO2. Each culture normally contains at least 5 x 106 cells by the time of treatment termination. After treatment, cells were washed with calcium and magnesium free phosphate buffered saline, trypsinized and suspended in medium. Cell suspensions from each dose level were counted by Coulter Counter® and replated at about 1.5 x 106 cells into each of two 150-mm dishes and approximately 200 cells into each of three 60 mm dishes. These 60-mm dishes were incubated for 6 days for colony development and determination of the cytotoxicity associated with each treatment (see Protocol Deviation). The large dishes were incubated for 7 days to permit growth and expression of induced mutants. The large dishes were subcultured every 2 to 3 days to maintain logarithmic growth. At each subculture, the cells from the two 150 mm dishes of each dose level were trypsinized, combined, counted, and reseeded at approximately 1.5 x 106 cells into each of two 150 mm dishes."

"At the end of the phenotypic expression period (7 days), each culture was reseeded at approximately 2 x 105 cells per 100 mm dish (12 dishes total) in mutant selection medium. Also, three 60 mm dishes were seeded at approximately 200 cells per dish in normal culture medium to determine the cloning efficiency of each culture. Cells were incubated for 7 days at 35-38°C in a humidified incubator with 4-6% CO2."

Activation Assay
"The activation assay was performed concurrently with its own set of vehicle and positive controls. The procedure for this assay was identical to the assays performed without metabolic activation except for the addition of the S9 mix during the 4-hour treatment period. The fetal bovine serum content of the medium used during treatment was reduced to 5%."

3. Evaluation criteria:

A test substance is concluded to induce a positive response if (a) the MF is significantly different from the MF of the concurrent vehicle controls at the 95% or 99% confidence levels; (b) a dose related or cytotoxicity-related increase in MF should be observed in both the initial and

confirmatory assays; (c) if an increase in the MF is observed near the highest testable cytotoxic dose and the number of mutant colonies is more than twice the value and (d) if the MF exceeded the MF (15 x 10^{-6}) required to compensate for random fluctuations in the MF.

4. Statistical analysis: The data were analyzed for statistical significance using tables by Kastenbaum and Bowman (1970).

10.1.3.1.1 II. RESULTS AND DISCUSSION

A. Analytical determinations: It was reported that actual concentrations were not determined in the study.

B. Preliminary (Dose range-finding) cytotoxicity test: The cell growth inhibition doses were tested in a range from 4.93- 2500 ug/mL in assays with and without activation. Based on the finding of total lethality at 2500 µg/mL-S9, accompanied by a clonal efficiency (CE) rate of 17% at 1250 µg/mL-S9 and total lethality at ≥1250 µg/mL+S9, the high levels selected for the initial trial were 1600 µg/mL-S9 and 1200 µg/mL +S9.

C. Mutagenicity assays: Data from the initial 4-hour exposure without or with S9 are summarized in Table 1 and 2 (Study Report Tables 3 and 4) and data from the confirmatory trial are presented in Tables 3 and 4 (Study Report Tables 5 and 6).

Initial trial: Concentrations of 1600 µg/mL-S9 and 1200 µg/mL +S9 were discarded because of severe cytotoxicity. As shown in Tables 1 and 2, percent relative population growth (RPG) was ≤10% (1400 µg/mL-S9) and ≤15% at 1000 µg/mL+S9. Isolated significant increases in the mutation frequency (MF) were noted at 400 µg/mL-S9 and at 400, 500, 600 and 1000 µg/mL+S9. These increases were neither dose-related nor replicate in the duplicate culture.

Confirmatory trial: Dose ranges for the confirmatory trial were 1100 to 1600 µg/mL –S9 and 700 to 1200 µg/mL +S9. There was no evidence of severe cytotoxicity in either S9-activated or nonactivated doses up to the highest levels tested. As shown in Tables 3 and 4, no significant increases in the MF were seen in the confirmatory nonactivated trial. With S9, isolated and significant increases in the MF were recorded for single cultures at 700, 800, 1000, and 1100 µg/mL. These responses were not observed at comparable doses in the initial trial, occurred in only 1 of 2 replicates and generally did not exceed the MF (15 x 10^{-6}) required to compensate for random fluctuations in the MF, and were, therefore, regarded as anomalous. By contrast, the positive controls (BrdU at 50 ug/mL-S9; MCA at 5 ug/mL+S9) induced the expected significant ($p \leq .05$) increases in the MF in both trials.

Table 1. Initial Mutation Assay without Metabolic Activation

Assay No.: 28298-0-435OECD Test Article: MON 52708 Treatment Date: 05/19/2006

Test Article	Survival to Treatment		Relative Population Growth (% of Control)	Mutant Colonies: Dish Number												Total Mutant Colonies	Absolute CE ± SD (%)	Mutant Frequency in 10^{-6} Units[a]
	Mean Colony Number ± SD	% Vehicle Control		1	2	3	4	5	6	7	8	9	10	11	12			
Vehicle Control[b]	124.7 ± 2.5	95.9	98.1	1	0	1	0	0	2	0	1	0	2	0	0	7	99.0 ± 4.3	2.9
Vehicle Control[b]	133.3 ± 4.9	104.1	101.8	0	0	0	1	1	0	2	0	0	2	0	2	8	108.0 ± 4.3	3.1
Positive Control[c]	93.0 ± 6.2	71.5	65.9	6	3	6	5	8	7	5	6	6	9	7	2	64	86.7 ± 2.5	30.8**
Positive Control[c]	81.0 ± 4.0	62.3	81.0	4	5	6	4	3	6	3	3	5	3	5	3	50	107.7 ± 7.3	19.3**
Test Article (µg/mL)																		
400	115.0 ± 5.6	88.5	80.0	0	1	1	2	1	0	0	2	1	1	1	0	10	88.7 ± 14.2	4.7
400	121.0 ± 3.0	93.1	78.9	0	2	2	1	1	1	1	1	2	0	4	1	16	106.2 ± 6.5	6.3*
600	113.7 ± 6.1	88.0	67.7	0	1	0	0	0	1	0	1	0	0	0	1	4	91.2 ± 3.8	1.8
600	113.3 ± 7.4	87.2	86.9	0	1	2	0	1	2	2	1	1	0	1	1	12	108.7 ± 1.3	4.6
800	108.7 ± 11.7	83.6	69.7	0	0	0	1	0	0	1	0	1	1	0	0	4	107.2 ± 3.3	1.6
800	87.3 ± 7.5	67.2	46.4	1	3	0	0	0	0	0	1	1	1	1	0	8	103.0 ± 17.7	3.2
1000	106.0 ± 7.2	81.5	50.9	1	1	1	0	0	0	1	0	0	0	1	0	5	122.5 ± 14.0	1.7
1000	106.7 ± 9.5	82.1	65.6	1	0	0	1	0	0	0	0	2	1	0	0	5	108.3 ± 6.3	2.1
1200	86.0 ± 9.0	66.2	21.5	1	0	0	0	0	1	1	0	0	1	1	0	5	89.2 ± 10.0	2.8
1200	100.0 ± 9.2	76.9	31.5	1	0	0	1	0	0	0	2	0	1	0	1	6	137.0 ± 11.3	1.8
1400	33.7 ± 9.8	25.9	11.1	0	0	0	0	1	0	1	0	0	1	0	0	3	104.0 ± 8.5	1.2
1400	23.7 ± 3.2	18.7	7.3	0	2	1	0	0	0	0	0	0	1	0	0	4	94.5 ± 5.8	1.8

[a] Mutant Frequency = Total mutant colonies / [(No. of dishes) x (2 x 105)x (absolute CE/100)]
[b]Vehicle Contol = 1% dimethyl sulfoxide
[c]Positive Control = 50 µg/mL 5-Bromo-2'-deoxyuridine CE = Cloning efficiency Cells seeded for analysis: 200/dish for CE; 2 x 105/dish for mutants
* Significant increase: Kastenbaum Bowman test $p \leq 0.05$ but mutant frequency <15 x 10-6
** Significant increase: Kastenbaum Bowman test $p \leq 0.01$ and mutant frequency ≥15 x 10
Source: Study Report Table 3, p27 (MRID 47899512)

Table 2: Initial Mutation Assay with Metabolic Activation

Assay No.: 28298-0-435OECD Test Article: MON 52708 Treatment Date: 05/19/2006

Test Article	Survival to Treatment		Relative Population Growth (% of Control)	Mutant Colonies: Dish Number												Total Mutant Colonies	Absolute CE ± SD (%)	Mutant Frequency in 10^{-6} Units[a]
	Mean Colony Number ± SD	% Vehicle Control		1	2	3	4	5	6	7	8	9	10	11	12			
Vehicle Control[b]	130.7 ± 5.5	85.3	92.0	1	0	1	0	2	1	2	0	0	0	0	0	7	106.5 ± 9.1	2.7
Vehicle Control[b]	175.7 ± 5.1	114.7	107.6	0	0	2	0	0	0	2	0	0	0	0	1	5	98.5 ± 4.5	2.1
Positive Control[c]	253.3 ± 21.6	153.6	44.2	28	41	26	24	23	20	23	25	18	21	23	23	299	96.0 ± 7.5	129.8***
Positive Control[c]	132.0 ± 21.0	86.2	40.4	22	29	21	17	33	32	28	24	31	28	31	28	324	123.7 ± 12.3	109.2***
Test Article (µg/mL)																		
400	102.7 ± 13.5	67.0	158.5	1	0	0	0	0	0	0	2	0	0	0	1	4	82.7 ± 2.6	2.0
400	134.3 ± 11.0	81.2	153.8	2	1	3	0	0	3	2	0	1	2	1	0	15	71.3 ± 8.7	8.8**
500	88.0 ± 4.0	57.1	165.5	0	0	1	0	2	0	1	0	2	1	0	0	7	79.5 ± 4.8	3.7
500	202.3 ± 8.3	132.1	146.9	0	1	2	1	1	1	2	2	1	3	0	1	15	101.0 ± 8.0	6.2*
600	127.7 ± 6.7	102.8	97.9	2	0	2	1	0	3	1	0	1	1	2	1	14	138.5 ± 22.1	4.2
600	185.7 ± 10.1	69.0	74.3	0	4	1	1	0	1	1	0	1	1	2	1	13	83.0 ± 3.5	6.5*
700	83.7 ± 8.0	54.6	68.0	2	1	1	1	0	0	2	2	1	2	0	0	12	131.5 ± 15.2	3.8
700	119.7 ± 30.6	78.1	83.4	1	1	0	0	1	0	0	0	0	0	0	3	6	100.2 ± 9.6	2.5
800	74.0 ± 8.7	48.3	61.1	1	0	0	0	0	3	0	0	0	0	2	1	7	104.0 ± 7.5	2.8
800	135.0 ± 29.1	88.1	89.7	0	0	1	1	1	1	2	1	0	1	0	0	8	124.7 ± 8.0	2.7
1000	47.7 ± 6.7	31.1	14.3	0	3	0	1	0	1	0	0	0	0	0	0	5	102.5 ± 9.7	2.0
1000	64.3 ± 7.4	42.0	15.7	2	0	1	1	1	2	3	0	2	2	2	3	19	113.8 ± 5.8	7.0**

[a] Mutant Frequency = Total mutant colonies / [(No. of dishes) x (2 x 105)x (absolute CE/100)]
[b]Vehicle Contol = 1% dimethyl sulfoxide
[c]Positive Control = 5 µg/mL 3-methylcholanthrene CE = Cloning efficiency Cells seeded for analysis: 200/dish for CE; 2 x 105/dish for mutants
* Significant increase: Kastenbaum Bowman test p ≤0.05 but mutant frequency <15 x 10-6
** Significant increase: Kastenbaum Bowman test p ≤0.01 but mutant frequency <15 x 10-6
*** Significant increase: Kastenbaum Bowman test p ≤0.01 and mutant frequency ≥15 x 10-6
Source: Study Report Table 4 p28 (MRID 47899512)

Table 3: Confirmatory Mutation Assay without Metabolic Activation

Assay No.: 28298-0-435OECD Test Article: MON 52708 Treatment Date: 06/09/2006

Test Article	Survival to Treatment		Relative Population Growth (% of Control)	Mutant Colonies: Dish Number												Total Mutant Colonies	Absolute CE ± SD (%)	Mutant Frequency in 10^{-6} Units[a]
	Mean Colony Number ± SD	% Vehicle Control		1	2	3	4	5	6	7	8	9	10	11	12			
Vehicle Control[b]	173.3 ± 12.1	85.9	101.0	0	2	1	2	0	2	2	1	1	1	2	1	15	103.7 ± 4.3	6.0
Vehicle Control[b]	232.7 ± 13.6	114.1	95.8	0	0	1	1	2	0	0	2	0	1	3	1	11	131.7 ± 9.7	3.5
Positive Control[c]	99.0 ± 10.6	48.5	43.9	11	12	5	7	4	10	6	10	5	11	8	7	96	101.3 ± 4.3	39.3*
Positive Control[c]	110.3 ± 11.0	54.1	61.1	18	10	11	9	5	12	7	11	13	8	11	8	123	85.2 ± 2.5	53.9*
Test Article (µg/mL)																		
1100	160.0 ± 16.7	78.4	64.5	0	1	0	3	0	3	2	3	1	1	1	1	16	120.3 ± 7.2	5.5
1100	250.0 ± 16.5	122.5	80.6	0	1	4	0	0	1	1	2	0	1	3	2	15	103.3 ± 14.1	6.0
1200	224.7 ± 21.8	110.1	73.1	1	2	0	1	0	4	1	2	0	1	0	1	13	94.5 ± 8.2	5.7
1200	252.7 ± 10.6	123.9	83.2	0	1	1	0	0	1	2	0	0	1	2	1	9	103.8 ± 5.4	3.6
1300	230.0 ± 16.4	112.7	56.6	1	2	2	1	3	3	0	1	2	0	2	1	17	90.3 ± 10.9	7.8
1300	146.7 ± 9.3	71.9	59.7	0	3	1	1	2	3	1	0	2	1	2	0	16	103.0 ± 0.5	6.5
1400	121.3 ± 18.8	59.5	59.7	1	0	1	2	1	0	1	3	3	0	0	1	13	103.7 ± 7.6	5.1
1400	223.0 ± 9.5	109.3	56.9	2	2	1	2	2	0	0	2	1	3	1	2	18	149.0 ± 8.7	5.0
1500	116.7 ± 8.1	57.2	45.3	0	2	1	0	4	2	1	0	2	2	2	3	19	160.8 ± 3.9	7.9
1500	125.7 ± 8.7	61.6	40.8	3	1	2	0	1	2	1	0	1	1	2	0	14	122.7 ± 4.3	4.8
1600	53.3 ± 4.2	26.1	34.3	1	1	1	1	1	2	3	1	3	1	2	0	17	112.8 ± 2.5	6.3
1600	68.0 ± 1.7	33.3	21.4	0	1	1	1	2	1	0	1	1	0	1	0	9	80.2 ± 12.3	4.7

[a] Mutant Frequency = Total mutant colonies / [(No. of dishes) x (2 x 105)x (absolute CE/100)]
[b]Vehicle Contol = dimethyl sulfoxide
[c]Positive Control = 50 µg/mL 5-Bromo-2'-deoxyuridine CE = Cloning efficiency Cells seed
Source: Study Report Table 5, p29 (MRID 47899512)

Table 4: Confirmatory Mutation Assay with Metabolic Activation

Assay No.: 28298-0-435OECD Test Article: MON 52708 Treatment Date: 06/09/2006

Test Article	Survival to Treatment		Relative Population Growth (% of Control)	Mutant Colonies/Dish Number												Total Mutant Colonies	Absolute CE ± SD (%)	Mutant Frequency in 10⁻⁶ Units[a]
	Mean Colony Number ± SD	% Vehicle Control		1	2	3	4	5	6	7	8	9	10	11	12			
Vehicle Control[b]	208.3 ± 11.2	97.5	87.3	0	1	3	1	0	2	0	3	1	1	0	0	12	97.2 ± 11.9	5.1
Vehicle Control[b]	219.0 ± 3.5	102.5	112.8	0	1	1	2	1	1	2	0	1	0	0	2	11	86.0 ± 3.9	5.3
Positive Control[c]	153.0 ± 8.1	71.6	57.4	36	33	30	40	42	44	36	42	38	39	35	33	488	95.0 ± 4.6	214.0***
Positive Control[c]	134.0 ± 16.1	62.7	57.7	48	30	43	51	47	47	42	34	32	41	46	39	540	79.0 ± 6.6	284.8***
Test Article (µg/mL)																		
700	230.0 ± 10.4	117.0	100.9	3	4	2	4	1	4	0	2	1	2	2	1	26	99.7 ± 7.0	10.9**
700	163.0 ± 16.7	76.3	68.5	2	1	1	0	0	2	1	4	1	1	1	0	14	88.8 ± 24.5	6.6
800	194.7 ± 11.6	91.1	83.1	0	4	1	1	1	3	1	0	3	1	1	3	19	86.0 ± 5.6	9.2*
800	317.0 ± 21.0	148.4	90.5	1	3	0	0	2	1	0	1	0	1	1	1	11	88.8 ± 6.5	5.2
900	269.0 ± 18.4	125.9	86.5	1	1	2	4	2	1	1	0	1	2	1	1	17	98.7 ± 9.8	7.2
900	217.7 ± 13.5	101.9	90.6	0	0	0	1	0	1	1	1	1	1	0	0	6	94.8 ± 3.3	2.6
1000	311.0 ± 22.5	145.6	61.8	0	0	3	3	1	2	2	1	2	3	1	0	18	90.0 ± 3.0	8.3
1000	141.7 ± 8.7	66.3	53.7	1	5	5	2	1	3	2	6	7	6	5	1	44	82.8 ± 6.9	22.4***
1100	116.0 ± 11.5	54.3	18.7	0	0	0	1	0	0	2	0	0	0	1	C	4	91.8 ± 7.6	2.0
1100	127.0 ± 6.9	59.4	18.4	4	1	1	2	1	4	3	4	6	1	1	1	29	77.3 ± 2.8	15.6***
1200	72.3 ± 10.6	33.9	6.8	0	2	0	2	0	0	0	1	0	3	2	0	10	81.2 ± 3.3	5.1
1200	50.7 ± 6.0	23.7	8.7	0	0	0	1	2	0	2	2	4	1	1	3	16	70.0 ± 6.5	9.5

[a] Mutant Frequency = Total mutant colonies / [(No. of dishes) x (2 x 105)x (absolute CE/100)]
[b]Vehicle Contol = 1% dimethyl sulfoxide
[c]Positive Control = 5 µg/mL 3-methylcholanthrene C = contaminated Cells seeded for analysis: 200/dish for CE; 2 x 105/dish for mutants
* Significant increase: Kastenbaum Bowman test p ≤0.05 but mutant frequency <15 x 10-6
** Significant increase: Kastenbaum Bowman test p ≤0.01 but mutant frequency <15 x 10-6
*** Significant increase: Kastenbaum Bowman test p ≤0.01 and mutant frequency ≥15 x 10
Source: Study Report Table 6, p30 (MRID 47899512)

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

10.1.3.1.1.1 B. REVIEWER'S COMMENTS

RELIABILITY RATING: Totally reliable
This study is generally compliant with OECD 476 (1997)

C. CONCLUSIONS: MON 52708 was tested up severely cytotoxic concentrations (1600 µg/mL –S9; 1200 µg/mL +S9) but failed to induce a mutagenic effect. Isolated significantly increased MFs (700, 800, 1000 and 1100 ug/mL) were seen in the presence of S9 activation. However, these responses were generally not observed at comparable doses in the initial trial, occurred in only 1 of 2 replicates and generally did not exceed the MF (15 x 10^{-6}) required to compensate for random fluctuations in the MF, and were, therefore, regarded as anomalous. We agree with this assessment. The expected responses were obtained with the negative and positive controls either with or without S9 activation. It was, therefore, concluded that MON 52708 was negative in this test system in a well-conducted study.

Deficiencies: None.

Revised by U.S. Environmental Protection Agency

In Vitro Gene Mutation assay in Chinese hamster cells HGPRT; OCSPP 870.5300 [' 84-2]; OECD 476

Report: IIA 5.4.2/01. Cifone, M., (2007). CHO HGPRT forward mutation assay with a confirmatory assay and duplicate cultures with MON 11900. Covance Laboratories Inc., Vienna, Virginia, Monsanto Study No. CV-2006-082, April 18, 2007, unpublished, MRID 47899526.

Dates of work: August 21, 2006- October 27, 2006.

Guidelines: OPPTS 870. 5300 (August, 1998)
OECD 476 (July 21, 1997)
JMAFF –(Nohsan No .6283, Shirasu, 1988))

GLP: Yes USEPA Principles of GLP, ENV/MC/CHEM (1989)
Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

GLP: Exceptions None

EXECUTIVE SUMMARY:

In independently performed mammalian cell forward gene mutation assays (MRID 47899526), Chinese hamster ovary cells at the HGPRT locus were exposed for 4 hours to MON 11900 (Purity 94.6%; Lot/Batch No. GLP-0604-17184-T), prepared in dimethyl sulfoxide (DMSO), at concentrations of 0, 1200, 1400, 1600, 1800, 2000, and 2400 µg/mL+/-S9. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254.

Levels for the main assay were determined following a preliminary cytotoxicity test. Compound precipitation was only seen at 2400 µg/mL -S9 at the start of treatment but not at the end of exposure. No cytotoxicity was observed at any nonactivated concentration and only at 2400 µg/mL+S9. Accordingly, 2400 µg/mL+/-S9 was selected as the starting level for both trials of the mutation assay. This test level was in excess of the limit dose (10mM) for this test system.

In agreement with the preliminary findings, MON 11900 was not cytotoxic. Two isolated instances of significant increases in the mutation frequency (MF) were reported. In the initial trial, the MF rose to 7.0 and 7.5 X 10^{-6} mutants/survivors in 1 of 2 duplicate cultures at 1400 ug/mL -S9 and 1,600 ug/mL +S9, respectively. Neither of these increases were dose-related, replicated in the duplicate culture or confirmed in the repeat trial.

Based on these considerations, it was concluded that under the conditions of this study, MON 11900 did not induce forward mutations at the HGPRT locus in CHO cells with or without metabolic activation.

This study is classified as reasonably **totally reliable (acceptable/guideline)** and satisfies the guideline requirement (OCSPP 870.5300; OECD 476) for i*n vitro* mutagenicity (mammalian cell forward gene mutation) data.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: MON 11900

Description: White solid
Lot/Batch#: GLP-0604-17184-T
Purity: 94.6%
CAS #: Not given
Stability of test compound: Listed as stable until April 2007.

2. Control materials

Negative control: None
Solvent control: DMSO /1% (10 uL/mL)
Positive control: Non-activation: 5-Bromo-2' deoxyuridine (BrdU) at 50 ug/mL
Activation: 3-Methyl -cholanthrene (MCA) at 5 ug/mL

3. Activation: S9 derived from male Sprague-Dawley rats

X	Induced	X	Aroclor 1254	X	Rat	X	Liver
	Non-induced		Phenobarbital		Mouse		Lung
			β-naphthoflavone		Hamster		Other
			Other		Other		

The S9 fraction was prepared commercially and the S9 mix contained the following components:

S9 Mix Components

Component	Amount
NADP (sodium salt)	0.8 mM
Glucose-6-phosphate	1.0 mM
Calcium chloride	2.0 mM
Potassium chloride	6.0 mM
Magnesium Chloride	2.0 mM
Phosphate	10.0 mM
S9 homogenate	10.0 uL/mL

4. Test cells: Mammalian cells in culture

	Mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
X	Chinese hamster ovary (CHO) cells		list any others
	Human peripheral lymphocytes		

Properly maintained?	X	Yes		No
Periodically checked for Mycoplasma contamination?	X	Yes		No
Periodically checked for karyotype stability?	X	Yes		No

Media: Ham' Nutrient Mixture supplemented with L-glutamine , gentamicin, Fungizone, and 8% v/v fetal bovine serum .

5. **Locus examined**

		Thymidine kinase (TK)	X	**Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)**		**Na^+/K^+ ATPase**
Selection agent:		Bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		Ouabain
		Fluorodeoxyuridine (FdU)	X	6-thioguanine (6-TG; 24μ M)		
	X	Trifluorothymidine (TFT)				

6. **Test compound concentrations used:**

b. Preliminary cytotoxicity (triplicate plates from single culture for each test group)

	Concentrations tested (μg/mL)										
4 hours, without S9	0	4.70	9.40	18.8	37.5	75.0	150	300	600	1200	2400
4 hours, with S9	0	4.70	9.40	18.8	37.5	75.0	150	300	600	1200	2400

b. Mutagenicity assay (triplicate plates for survival & 12 plates for mutant selection from duplicate cultures per test group)—both trials

	Concentrations tested (μg/mL)						
4 hours, without S9	0	1200	1400	1600	1800	2000	2400
4 hours, with S9	0	1200	1400	1600	1800	2000	2400

B. STUDY DESIGN AND METHODS

1. In life dates: August 21, 2006 – October 27 2006.

2. Treatment:

Preliminary cytotoxicity assay: Concentrations for the main study were determined from the results of a preliminary cell growth inhibition test in which dishes of 200 cells each (3 dishes/concentration) were exposed to a series of 10 levels of the test material for 4 hours, with and without S9,in a 4-6% CO2 atmosphere. The cells were washed with Dublbecco's phosphate buffered saline and then incubated in F12 culture medium for 6 days. The dishes were washed and the colonies were fixed in methanol and stained with Giemsa and counted manually. Cytotoxicity was expressed as a percentage of mean counts for each level as compared to the vehicle controls.

Mutation assay: As stated in the study report:

Nonactivation Assay
"The cleansed cells were plated at 4 x 106 cells per T-75 (75 cm2) tissue culture flask on the day before dosing. At the time of dosing, cell cultures were treated with the test or control article for about 4 hours at 35-38□C in a humidified atmosphere with 4-6% CO2. Each culture normally contains at least 5 x 106 cells by the time of treatment termination. After treatment, cells were washed with calcium and magnesium free phosphate buffered saline, trypsinized and suspended in medium. Cell suspensions from each dose level were counted by Coulter Counter and replated at about 1.5 x 106 cells into each of two 150-mm dishes and approximately 200 cells into each of three 60 mm dishes. These 60-mm dishes were incubated for 6 days for colony development and determination of the cytotoxicity associated with each treatment (see Protocol Deviation). The large dishes were incubated for 7 days to permit growth and expression of induced mutants. The large dishes were subcultured every 2 to 3 days to maintain logarithmic growth. At each subculture, the cells from the two 150 mm dishes of each dose level were trypsinized, combined, counted, and reseeded at approximately 1.5 x 106 cells into each of two 150 mm dishes."

"At the end of the phenotypic expression period (7 days), each culture was reseeded at approximately 2 x 105 cells per 100 mm dish (12 dishes total) in mutant selection medium. Also, three 60 mm dishes were seeded at approximately 200 cells per dish in normal culture medium to determine the cloning efficiency of each culture. Cells were incubated for 6 days at 35-38□C (see Protocol Deviation) in a humidified incubator with 4-6% CO2."

Activation Assay
"The activation assay was performed concurrently with its own set of vehicle and positive controls. The procedure for this assay was identical to the assays performed without metabolic activation except for the addition of the S9 mix during the 4-hour treatment period. The fetal bovine serum content of the medium used during treatment was reduced to 5%."

3. Evaluation criteria:

Assay validity: The assay was considered valid if: (a) the average cloning efficiency (CE) of the vehicle control was between 50 and 115%; (b) the background MF was 0 to $10x10^{-6}$ and (c) the positive control induced a MF that was significantly ($p \leq 0.01$) higher that the vehicle control.

Positive response: A test substance is concluded to induce a positive response if (a) the MF is significantly different from the concurrent vehicle controls at the 95% or 99% confidence levels and was dose related and (b) if the test material induces MFs in excess of 15×10^{-6} to compensate for random fluctuations in the MF.

4. Statistical analysis: The data were analyzed for statistical significance using tables by Kastenbaum and Bowman (1970).

II. RESULTS AND DISCUSSION

A. Analytical determinations: It was reported that actual concentrations were not determined in the study.

B. Preliminary (Dose range-finding) cytotoxicity test: Compound precipitation was only seen at 2400 µg/mL -S9 at the start of treatment but not at the end of exposure. Cytotoxicity was evaluated over a range of 4.70- 2400 ug/mL with or without activation. Cytotoxicity was not seen in levels of 1250 and 2400 ug/mL in assays without activation. Severe cytotoxicity was reported only at 2400 ug/mL +S9, with no cells surviving treatment (See Study Report, Tables 1 and 2).

Table 1: Dose Range Finding Assay without Metabolic Activation

Assay No.: 28354-0-435OECD
Test Article: MON 11900
Treatment Date: 09/07/2006

Applied Concentration µg/mL	Number of Colonies Dish #1	Dish #2	Dish #3	Average Count	Relative Cloning Efficiency (%)[a]	Absolute Cloning Efficiency (%)[b]
VC[c]	222	215	223	220.0	100.0	110.0
4.70	NC	NC	NC	-	-	
9.40	NC	NC	NC	-	-	
18.8	NC	NC	NC	-	-	
37.5	NC	NC	NC	-	-	
75.0	NC	NC	NC	-	-	
150	NC	NC	NC	-	-	
300	NC	NC	NC	-	-	
600	NC	NC	NC	-	-	
1200	239	250	227	238.7	108.5	
2400	203	244	202	216.3	98.3	

[a]Relative to vehicle control cloning efficiency for all treatments.
[b]Average count divided by the number of cells plated (200) x 100%. The absolute cloning efficiency was
determined for the vehicle control cultures to assess the viability of the cultures.
[c]VC = Vehicle control; DMSO, 10 □L/mL
NC = Not counted
Source: Study Report, Table 1, p. 26 (MRID 47899526)

Table 2: Dose Range Finding Assay with Metabolic Activation

Assay No.: 28354-0-435OECD
Test Article: MON 11900
Treatment Date: 09/07/2006

Applied Concentration ug/mL	Number of Colonies Dish #1	Dish #2	Dish #3	Average Count	Relative Cloning Efficiency (%)[a]	Absolute Cloning Efficiency (%)[b]
VC[c]	224	240	202	222.0	100.0	111.0
4.70	NC	NC	NC	-	-	
9.40	NC	NC	NC	-	-	
18.8	NC	NC	NC	-	-	
37.5	NC	NC	NC	-	-	
75.0	NC	NC	NC	-	-	
150	NC	NC	NC	-	-	
300	NC	NC	NC	-	-	
600	185	212	189	195.3	88.0	
1200	205	232	203	213.3	96.1	
2400	0	0	0	0.0	0.0	

[a]Relative to vehicle control cloning efficiency for all treatments.
[b]Average count divided by the number of cells plated (200) x 100%. The absolute cloning efficiency was
determined for the vehicle control cultures to assess the viability of the cultures.
[c]VC = Vehicle control; DMSO, 10 □L/mL
NC = Not counted
Source: Study Report, Table 2, p. 27 (MRID 47899526)
Source: Table2, p. 27

C. Mutagenicity assays: Data from the 4-hour exposure without or with S9 are summarized in Tables 3 and 4 (Initial trial) and Tables 5 and 6 of the study report (Confirmatory trial).

Initial trial: As shown in Study Report Tables 3, and 4, the test material was not cytotoxic at any concentration with or without S9 activation. MFs of single cultures ($7.0x10^{-6}$ at 1400 ug/mL-S9 and $7.0x10^{-6}$ at 1600 ug/mL +S9) were significantly increased ($p \leq .01$ and 0.05, respectively). However, the increases were limited to these cultures and not dose-related. These findings were, therefore, considered to be spurious.

Confirmatory trial: Comparable test levels were assayed in the confirmatory assay. There was no evidence of cytotoxicity in either S9-activated or nonactivated doses up to the highest level. Additionally, there were no increases in the MF at any nonactivated or S9-activated concentration. By contrast, the positive controls (BrdU at 50 ug/mL-S9; MCA at 5 ug/mL+S9) induced the expected significant ($p \leq .05$) increases in the MF in both trials.

Table 3: Initial Mutation Assay without Metabolic Activation

Assay No.: 28354-0-435OECD Test Article: MON 11900 Treatment Date: 09/22/2006

Test Article	Survival to Treatment: Mean Colony Number ± SD	Survival to Treatment: % Vehicle Control	Relative Population Growth (% of Control)	Mutant Colonies/ Dish Number: 1	2	3	4	5	6	7	8	9	10	11	12	Total Mutant Colonies	Absolute CE ± SD (%)	Mutant Frequency in 10^{-6} Units[a]
Vehicle Control[b]	154.7 ± 7.5	108.7	106.0	1	0	1	3	1	1	2	1	2	0	0	0	12	130.7 ± 9.9	3.8
Vehicle Control[b]	135.3 ± 25.5	95.3	84.0	0	0	0	1	0	2	0	0	1	0	0	1	5	112.8 ± 10.7	1.8
Positive Control[c]	85.0 ± 6.1	44.8	54.3	3	2	3	3	3	4	3	3	3	2	3	3	43	104.8 ± 3.6	17.1*
Positive Control[c]	88.7 ± 16.9	61.1	46.2	2	7	6	4	4	6	2	2	5	1	2	1	42	84.2 ± 5.3	20.8*
Test Article (µg/mL)																		
1200	105.0 ± 4.4	72.4	115.5	1	1	1	3	0	1	0	0	0	0	1	1	9	68.2 ± 8.5	5.5
1200	173.3 ± 8.1	119.5	96.7	1	1	0	1	1	1	1	0	1	2	0	0	9	120.8 ± 21.5	3.1
1400	183.0 ± 3.6	126.2	71.3	0	2	0	2	0	4	1	3	1	3	0	5	20	118.5 ± 12.3	7.0**
1400	118.3 ± 19.7	81.6	87.1	2	0	0	0	1	0	2	0	2	0	1	0	8	109.5 ± 5.7	3.0
1600	143.0 ± 17.4	98.6	103.1	0	1	0	3	0	0	0	3	1	2	0	1	11	105.0 ± 8.5	4.4
1600	124.0 ± 19.1	85.5	88.9	2	0	0	0	0	0	0	0	0	0	1	0	3	117.8 ± 3.8	1.1
1800	159.7 ± 8.0	110.1	81.1	2	0	4	1	0	0	1	0	2	1	1	2	14	118.2 ± 9.2	4.9
1800	132.7 ± 14.8	91.5	89.6	0	0	0	0	0	1	0	0	1	0	0	0	2	125.8 ± 8.3	0.7
2000	131.7 ± 10.4	90.8	72.6	0	0	1	0	3	0	1	0	0	1	1	1	8	114.5 ± 10.1	2.9
2000	142.7 ± 8.7	98.4	103.1	0	0	1	0	0	1	0	0	0	0	0	0	2	121.2 ± 9.6	0.7
2400	133.3 ± 11.1	90.6	105.2	0	2	1	0	0	1	0	0	0	0	0	1	5	118.8 ± 13.2	1.8
2400	142.3 ± 17.2	98.2	82.4	1	0	1	0	0	0	1	0	1	0	0	0	4	118.2 ± 14.8	1.4

[a] Mutant Frequency = Total mutant colonies / [(No. of dishes) x (2 x 105)x (absolute CE/100)]

[b]Vehicle Contol = dimethylsulfoxide, 10 □L/mL

[c]Positive Control = 50 □g/mL 5-Bromo-2'-deoxyuridine

CE = Cloning efficiency Cells seeded for analysis: 200/dish for CE; 2 x 105/dish for mutants

* Significant increase: Kastenbaum Bowman test $p \leq 0.01$ and mutant frequency $\geq 15 \times 10\text{-}6$

** Significant increase: Kastenbaum Bowman test $p \leq 0.01$ but mutant frequency $< 15 \times 10\text{-}6$

Source: Study Report, Table 3, p. 28 (MRID 47899526)

Table 4: Initial Mutation Assay with Metabolic Activation

Assay No.: 28354-0-435OECD Test Article: MON 11900 Treatment Date: 09/22/2006

Test Article	Survival to Treatment: Mean Colony Number ± SD	Survival to Treatment: % Vehicle Control	Relative Population Growth (% of Control)	Mutant Colonies/Dish Number: 1	2	3	4	5	6	7	8	9	10	11	12	Total Mutant Colonies	Absolute CE ± SD (%)	Mutant Frequency in 10^{-6} Units[a]
Vehicle Control[b]	160.3 ± 16.5	104.0	114.4	0	1	2	1	0	0	0	1	0	0	0	1	6	76.7 ± 9.5	3.8
Vehicle Control[b]	148.0 ± 6.1	96.0	83.0	0	0	0	1	0	1	0	0	0	1	1	1	5	78.7 ± 3.0	2.6
Positive Control[c]	197.7 ± 6.4	128.2	68.0	71	63	53	37	48	57	38	43	55	59	38	46	632	90.2 ± 2.8	292.1*
Positive Control[c]	220.3 ± 12.9	142.9	65.0	55	57	49	49	54	43	50	58	66	51	72	53	657	79.2 ± 8.6	345.8*
Test Article (µg/mL)																		
1200	146.3 ± 8.3	94.9	102.2	1	0	1	2	0	0	0	0	1	1	0	0	6	76.7 ± 3.5	3.3
1200	199.7 ± 34.2	129.5	54.1	1	0	2	0	0	1	0	0	0	0	1	3	8	73.3 ± 2.6	4.5
1400	149.0 ± 19.7	96.6	93.6	0	0	1	0	0	1	1	0	0	0	0	0	3	90.3 ± 1.7	1.4
1400	139.7 ± 8.3	90.6	60.0	0	0	0	1	0	0	0	1	1	0	0	1	4	84.2 ± 6.4	2.0
1600	144.3 ± 16.3	93.6	88.6	1	0	1	0	0	0	0	0	1	1	0	0	4	65.5 ± 2.3	2.5
1600	128.7 ± 3.0	83.5	105.1	0	1	1	5	2	1	0	2	1	2	1	1	17	94.3 ± 2.8	7.5**
1800	135.7 ± 4.7	88.5	87.6	0	0	0	0	1	0	0	0	1	0	0	1	3	108.3 ± 12.3	1.2
1800	155.7 ± 22.0	101.0	82.1	1	0	0	2	1	1	1	1	1	2	0	0	10	131.5 ± 1.3	3.2
2000	176.0 ± 9.2	114.2	63.1	2	0	0	0	1	1	0	0	1	0	3	0	8	112.3 ± 2.0	3.0
2000	153.3 ± 7.5	100.8	61.1	2	1	0	0	3	1	1	0	0	0	0	0	8	104.5 ± 13.1	3.2
2400	137.3 ± 17.6	89.1	83.0	0	0	0	0	0	0	0	0	0	1	0	0	1	112.5 ± 3.9	0.4
2400	154.3 ± 12.6	100.1	109.4	0	0	0	0	0	0	0	0	0	1	0	0	1	83.0 ± 3.8	0.5

[a] Mutant Frequency = Total mutant colonies / [(No. of dishes) x (2 x 105)x (absolute CE/100)]

[b]Vehicle Contol = dimethylsulfoxide, 10 □L/mL

[c]Positive Control = 5 □g/mL 3-methylcholanthrene

CE = Cloning efficiency Cells seeded for analysis: 200/dish for CE; 2 x 105/dish for mutants

* Significant increase: Kastenbaum Bowman test p ≤0.01 and mutant frequency ≥15 x 10-6

** Significant increase: Kastenbaum Bowman test p ≤0.05 but mutant frequency <15 x 10-6

Source: Study Report, Table 4, p. 29 (MRID 47899526)

Table 5: Confirmatory Mutation Assay without Metabolic Activation

Assay No.: 28354-0-435OECD Test Article: MON 11900 Treatment Date: 10/13/2006

Test Article	Survival to Treatment: Mean Colony Number ± SD	Survival to Treatment: % Vehicle Control	Relative Population Growth (% of Control)	Mutant Colonies Dish Number: 1	2	3	4	5	6	7	8	9	10	11	12	Total Mutant Colonies	Absolute CE ± SD (%)	Mutant Frequency in 10⁻⁶ Units[a]
Vehicle Control[b]	187.0 ± 9.2	100.4	96.5	3	0	0	1	0	2	1	2	2	1	0	1	13	87.8 ± 9.7	6.2
Vehicle Control[b]	185.7 ± 13.2	99.6	103.3	1	1	1	0	0	0	2	0	3	0	0	0	8	93.5 ± 2.3	3.6
Positive Control[c]	98.3 ± 13.6	52.7	53.4	2	3	4	11	7	2	4	8	3	2	5	8	61	79.3 ± 8.9	32.0*
Positive Control[c]	98.3 ± 3.5	52.8	35.5	4	5	5	4	2	5	3	4	6	3	0	1	42	78.7 ± 7.8	22.2*
Test Article (µg/mL)																		
1200	198.0 ± 11.4	106.3	94.9	1	0	0	0	0	2	0	0	0	0	1	4	8	83.2 ± 6.4	4.0
1200	193.7 ± 5.7	103.9	95.5	1	0	1	1	2	0	1	2	1	0	0	0	9	86.5 ± 3.3	3.7
1400	186.7 ± 9.5	89.4	78.3	2	2	0	2	1	3	0	1	1	0	0	1	13	78.0 ± 0.3	7.7
1400	176.3 ± 11.9	94.6	69.8	0	1	1	1	2	0	1	2	0	1	1	2	12	70.7 ± 8.0	7.1
1600	169.7 ± 10.7	91.1	80.2	1	0	0	0	0	1	0	0	2	1	0	3	8	75.0 ± 9.8	4.4
1600	181.7 ± 6.0	97.5	94.3	1	0	1	0	3	1	1	1	3	0	2	0	13	83.8 ± 11.1	6.3
1800	134.0 ± 8.7	82.6	75.7	3	1	0	0	0	0	2	1	0	0	0	1	8	80.8 ± 11.4	4.1
1800	167.7 ± 2.5	90.0	62.9	0	0	0	0	0	0	1	0	1	0	2	2	6	76.3 ± 3.8	3.3
2000	203.7 ± 14.5	109.3	85.8	1	1	3	1	2	0	0	0	0	2	1	1	12	72.3 ± 12.3	6.9
2000	186.0 ± 21.9	99.8	71.7	0	0	1	2	0	2	1	1	3	2	1	0	13	89.3 ± 9.0	5.5
2400	173.3 ± 9.5	93.0	83.2	0	0	1	1	2	1	0	0	0	0	0	0	5	88.0 ± 16.0	2.4
2400	178.0 ± 12.8	95.5	88.7	0	0	0	1	0	1	1	1	1	0	2	1	8	97.0 ± 3.0	3.4

[a] Mutant Frequency = Total mutant colonies / [(No. of dishes) x (2 x 105)x (absolute CE/100)]

[b] Vehicle Contol = dimethylsulfoxide, 10 □L/mL

[c] Positive Control = 50 □g/mL 5-Bromo-2'-deoxyuridine

CE = Cloning efficiency Cells seeded for analysis: 200/dish for CE; 2 x 105/dish for mutants

* Significant increase: Kastenbaum Bowman test $p \leq 0.01$ and mutant frequency ≥ 15 x 10-6

Source: Study Report, Table 5, p. 30 (MRID 47899526)

Table 6: Confirmatory Mutation Assay with Metabolic Activation

Assay No.: 28354-0-435OECD Test Article: MON 11900 Treatment Date: 10/13/2006

Test Article	Survival to Treatment: Mean Colony Number ± SD	Survival to Treatment: % Vehicle Control	Relative Population Growth (% of Control)	Mutant Colonies/Dish Number: 1	2	3	4	5	6	7	8	9	10	11	12	Total Mutant Colonies	Absolute CE ± SD (%)	Mutant Frequency in 10^{-6} Units[a]
Vehicle Control[b]	152.3 ± 10.7	89.8	84.7	1	1	0	1	2	1	2	1	1	1	0	3	14	88.0 ± 3.0	6.6
Vehicle Control[b]	187.0 ± 11.3	110.2	115.7	0	0	0	0	1	0	1	0	1	1	0	0	4	81.0 ± 4.9	2.1
Positive Control[c]	177.3 ± 19.1	104.5	84.0	50	44	46	54	45	53	48	38	52	50	38	51	571	57.2 ± 49.6	416.2*
Positive Control[c]	172.7 ± 22.0	101.8	91.6	47	49	49	38	48	53	51	49	47	52	42	41	566	77.3 ± 3.2	305.0*
Test Article (µg/mL)																		
1200	238.7 ± 10.8	152.5	108.4	1	1	0	1	1	0	1	0	0	0	2	2	9	96.8 ± 16.3	3.9
1200	266.7 ± 15.0	157.2	92.1	1	0	2	1	0	4	0	1	0	1	0	0	10	91.3 ± 6.3	4.6
1400	244.7 ± 12.1	144.3	105.3	1	0	0	1	0	1	1	2	1	1	1	1	10	67.5 ± 58.6	6.2
1400	232.0 ± 20.8	136.7	81.9	1	1	0	1	0	0	0	1	1	1	1	2	9	74.8 ± 6.8	3.8
1600	207.3 ± 17.9	122.2	97.8	0	1	1	0	0	0	0	0	1	1	0	2	6	98.5 ± 3.8	2.5
1600	219.0 ± 14.9	129.1	98.8	0	0	2	0	3	0	1	3	1	1	2	0	13	82.0 ± 8.5	6.6
1800	203.0 ± 11.3	119.6	61.8	1	0	0	0	0	2	0	0	0	0	0	0	3	77.8 ± 7.8	1.6
1800	186.3 ± 4.7	109.8	97.8	0	0	0	3	2	2	1	1	0	0	1	4	14	80.0 ± 5.8	7.3
2000	192.3 ± 13.3	113.4	103.1	0	0	2	1	1	0	0	0	0	3	2	2	11	86.8 ± 3.8	5.3
2000	186.0 ± 13.9	109.6	101.1	1	0	0	0	0	0	0	0	1	0	1	1	4	84.0 ± 8.3	2.0
2400	210.3 ± 4.5	124.0	120.4	1	0	0	1	3	1	0	1	0	1	0	2	10	99.0 ± 5.2	4.2
2400	197.3 ± 10.6	116.3	116.3	0	1	0	2	1	0	2	0	0	1	1	1	9	78.0 ± 80.7	3.4

[a] Mutant Frequency = Total mutant colonies / [(No. of dishes) x (2 x 105)x (absolute CE/100)]

bVehicle Contol = dimethylsulfoxide, 10 □L/mL

[c]Positive Control = 5 □g/mL 3-methylcholanthrene

CE = Cloning efficiency Cells seeded for analysis: 200/dish for CE; 2 x 105/dish for mutants

* Significant increase: Kastenbaum Bowman test $p \leq 0.01$ and mutant frequency $\geq 15 \times 10\text{-}6$

Source: Study Report Table 6, p31 (MRID 47899526)

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:
RELIABILITY RATING: Totally reliable
This study is compliant with OECD 476 (1997)

C. CONCLUSIONS: MON 11900 was tested up to an adequate high concentration 2400 ug/ml, which was in excess of the limit doses for this test system (10nM) but was not cytotoxic and failed to induce a mutagenic effect in either trial in the presence or absence of S9 activation. The responses induced by the positive controls (BrdU at 50 ug/mL-S9; MCA at 5 ug/mL+S9) indicate that the sensitivity of the assay was adequate to detect mutagenic activity. Based on these considerations, it was, therefore, concluded that MON 11900 was negative in this test system in a well-conducted study.

IIIA 10.1.4 Deficiencies: **None.**

Revised by U.S. Environmental Protection Agency

In vitro Mammalian Chromosome Aberration Test

Report: IIA 5.8/11. Murli, H. (2007). Chromosomal aberrations in cultured human peripheral blood lymphocytes with MON 52708. Covance Laboratories Inc., Monsanto report no. CV-2006-053; January 29, 2009; unpublished. MRID 47899510

Dates of work: May 2, 2006 – August 23, 2006

Guidelines: OECD 475, EPA OPPTS 870.5375, MAFF (Shirasu, 1988)
Deviations: None.
PMRA DACO 4.5.4

GLP: Yes OECD Principles of GLP, ENV/MC/CHEM (1998)
Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

GLP Exceptions: No

Executive Summary:

In an in vitro chromosome aberration test (MRID 47899510) cultured human lymphocytes were exposed to MON 52708 (Purity 97.9%, Lot/Batch no. GLP-0603-16958-T) prepared in dimethyl sulfoxide (DMSO), in independent experiments and were evaluated for clastogenic potential. On the basis of the assessment of reduced mitotic indices (MIs), the initial assay treatment period was 3 hours with a 22-hour harvest and was conducted with 0, 17.0, 24.2, 34.6, 49.4, 70.6, 101, 144, 206, 294, 420, 600, 858, 1230, 1750, and 2500 ug/mL +/-S9. Cultures treated with 0, 420, 600, 858, and 1230 ug/mL -S9 and 0, 294, 420, 600, and 858 ug/mL +S9 analyzed for chromosome aberrations.

In the confirmatory chromosomal assay, the treatment period was 22 hours without S9 and 3 hours with S9; cells were harvest at the end of the nonactivated exposure or 22 hours after the S9-activated exposure. The nonactivated phase of testing was repeated because of inadequate cytotoxicity for a valid test. The range of test concentrations was 62.5 to 700 ug/mL -S9 (Repeat test) and 250 to 1000 ug/mL +S9; cells exposed to 250, 350, 400 and 450 ug/mL -S9 and 750, 800, 850, 925, and 1000 ug/mL +S9 were scored for chromosome aberrations. The S9 homogenate was derived from the liver of rats induced with Aroclor 1254. Mitomycin C and cyclophosphamide served as the positive controls.

Compound precipitation was seen at ≥ 858 ug/mL +/- S9. MIs at the highest concentrations selected for analysis was 57% at 1230 ug/mL – S9 and 56% at 858 ug/mL + S9. No significant increase in cells with chromosomal aberration, polyploidy, or endoreduplication was observed in the initial nonactivated test. **With S9, the percentage of cells with aberrations was significantly ($p \leq 0.01$) increased at 858 ug/mL (9.5% versus 0 % in the vehicle and 0.5% in the culture**

medium); the predominate type of structural aberration was simple breaks; however, 2.0% of the scored cells also had chromatid exchanges, which is a complex aberration that is not commonly seen.
For the confirmatory trial, MIs at the highest concentration selected for analysis were 57% at 450 ug/mL – S9 and 55% at 1000 ug/mL + S9. Significant ($p \leq 0.01$) increases in structural chromosome aberrations of 4.0 and 4.5 % at 400 and 450 ug/mL – S9, respectively versus 0% in the vehicle and negative control groups were also scored in the nonactivated phase of testing. Significant ($p \leq 0.01$) increases in structural chromosome aberrations of 4.0 and 8.0 % at 800 and 1000 ug/mL + S9, respectively versus 0% in the vehicle and negative control groups were also scored. Although the study author dismissed these findings as either anomalous or indirectly associated with cytotoxicity, we disagree and believe that no conclusions can be reached. The testing of precipitating concentrations, the occurrence of significant results, while at different concentrations for both trials of the S9-activated tests and in 1 of 2 trials without S9, and the occurrence of complex aberrations in both S9-activated trials and in the repeat nonactivated trial render these data inconclusive.

Since a regulatory decision can not be made, this study is classified as **not reliable(unacceptable/non-guideline)** and does not satisfy the guideline requirement (OCSPP 870.5375, OECD 473) for in vitro mammalian cytogenetics (chromosome aberrations) data.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** DCSA (MON 52708)
   Description: White powder
   Lot/Batch#: GLP-0603-16958-T
   Purity: 97.9%
   CAS #: None given
   Stability of test compound: Expiration date listed as March 20, 2007

2. **Control materials**
   Negative control: Culture Medium (RPMI 1640)
   Solvent control: DMSO /1%
   Positive control: Non-activation: Mitomycin C (MMC) (0.75, 1.0, and 1.5 µg/mL, 3-hour treatment; 0.2, 0.3, and 0.4 µg/mL, ~22-hour treatment)
   Activation: Cyclophosphamide (CP, 200, 25.0, and 40.0 µg/mL)
   Both MMC and CP were dissolved in sterile, deionized water

3. **Activation**: S9 derived from rats (no further details provided)

X	Induced	X	Aroclor 1254	X	Rat	X	Liver
	Non-induced		Phenobarbital		Mouse		Lung
			β-naphthoflavone		Hamster		Other
			Other		Other		

The S9 fraction was obtained from (Molecular Toxicology, Inc. Aliquots of S9 were thawed immediately before use and added to the other components to form the activation system, as described below:

S9 Activation System

Component	Concentration in Cultures
NADP (sodium salt)	1.5 mg/mL (1.8 mM)
Isocitric acid	2.7 mg/mL (10.5 mM)
Homogenate (S9 fraction)	15.0 µL/mL (1.5%)

4. **Test cells:** Mammalian cells in culture

[ ]	Mouse lymphoma L5178Y cells	[ ]	V79 cells (Chinese hamster lung fibroblasts)
[ ]	Chinese hamster ovary (CHO) cells	[X]	Human whole blood lymphocytes

Properly maintained?	[X]	Yes	[ ]	No
Periodically checked for Mycoplasma contamination?	[ ]	Yes	Not reported	No
Periodically checked for karyotype stability?	[ ]	Yes	Not reported	No
Periodically "cleansed" against high spontaneous background?	[ ]	Yes	Not reported	No

Media: RPMI 1640 supplemented with HEPES buffer, approximately 20% heat-inactivated fetal bovine serum, penicillin (100 units/mL), streptomycin (100 µg/mL), L-glutamine (2 mM) and 2% phytohemagglutinin M

5. **Test compound concentrations used:**
   a. Preliminary cytotoxicity : Conducted in parallel with the cytogenetic assay.
   b. Cytogenetic assay: Duplicate cultures for each test dose, vehicle or positive control

Initial Trial:

Concentrations of MON 52708 tested (µg/mL)

3 hours, without S9	0	17.0	24.2	34.6	49.4	70.6	101	144	206	294	**420**	**600**	**858**	**1230**	1750	2500
3 hours, with S9	0	17.0	24.2	34.6	49.4	70.6	101	144	206	**294**	**420**	**600**	**858**	1230	1750	2500

Confirmatory Trial

Concentrations of MON 52708 tested (µg/mL)

22 hours, without S9	0	11.3	22.5	45.0	90.0	125	188	250	375	500	700	850	1000	1200
3 hours, with S9	0	250	375	500	700	**750**	**800**	**850**	**925**	**1000**				
22 hours, without S9 REPEAT	0	62.5	125	**250**	**350**	**400**	425	**450**	475	500	600	700		

Concentrations bolded were examined for chromosomal aberrations.

B. STUDY DESIGN AND METHODS

1. In life dates: May 24, 2006– August 23 2006.

2. Treatment:

Preliminary cytotoxicity assay: Cytotoxicity was determined in parallel with the cytogenetic assays by determining the mitotic index (MI), by counting the number of mitosis in 1000 cells.

Cytogenetic assay: Independently performed experiments were conducted.

a. **Cell exposure time:**

	Test material	Solvent control	Positive control
Non-activated:	3 h 22 h	3 h 22 h	3 h 22 h
Activated:	3 h	3 h	3 h

b. **Spindle inhibition:**

Inhibition used/concentration:	Colcemid/ 0.1 µg/mL
Administration time:	2 hours (before cell harvest)

c. **Cell harvest time after termination of treatment:**

	Test material	Solvent control	Positive control
Non-activated and Activated:	22, 0 & 22 h	22, 0 & 22 h	22, 0 & 22 h

d. **Details of slide preparation**: Following the colchicine treatment, cells were swollen with 75 mM KCl, fixed with a 3:1mixture of methanol and glacial acetic acid, and stained with 5% Giemsa. Two slides per group per experiment were prepared.

e. **Metaphase analysis**

No. of cells examined per dose: 200 (100 per duplicate culture in the treatment groups and the negative control. Only one culture from one of the positive control concentraions was scored.

Scored for structural?	x	Yes		No
Scored for numerical?	x	Yes, polyploidy cells and endoreduplication		No
Coded prior to analysis?	x	Yes		No

f. **Evaluation criteria:**

Assay validity: The assay is considered acceptable by the testing laboratory if (a) the negative and vehicle control cultures had >5% of the cells

with aberrations and the positive control induced a significant (≤0.01) increase in the number of chromosome aberrations.

Positive response: A test substance is concluded to induce a positive response if: the number of cells with structural chromosome aberrations was significantly (≤0.01) increase at one or more concentrations and the increase was dose-related.

g. **Statistical analysis**: The data were analyzed for statistical significance using the Cochran-Armitage test for linear trend and Fisher's exact test at $p \leq 0.01$.

II. RESULTS AND DISCUSSION

A. Analytical determinations: Analytical determinations were not performed.

B. Preliminary cytotoxicity test: An independent cytotoxicity test was not performed; cytotoxicity was measured in parallel with the cytogenetic assays.

C. Cytogenetic assays:

1. Initial assay: In the initial assay, compound precipitate was noted at ≥858 µg/mL +/-S9 and hemolysis was observed at harvest of the cultures treated with 2500 µg/mL +/-S9. Few if any cells survived treatment with ≥1750 µg/mL-S9 or ≥1230 µg/mL+S9. At 1230 µg/mL -S9, the MIs was reduced by 63% and at 858 µg/mL+S9 the MIs was reduced by 56%. Consequently, these concentrations were selected for the metaphase evaluation. Data from the cytogenetic evaluation are presented in Tables 1 and 2 for the nonactivated and S9-activated phase of testing (Study Report Tables 2 and 4, respectively). As shown, no significant increase in cells with structural chromosomal aberrations, polyploidy, or endoreduplication was observed at any nonactivated concentration of the test material. With S9, a significant (≤0.01) increase in the percentage of cells with aberrations (9.5%) was scored at 858 µg/mL; the main type of aberrations was simple breaks (6.5%); four chromatid exchanges (2%), which is a complex aberration, were also scored. The positive controls (MMC, 1.0 µg/mL-S9; CP 25.0 µg/mL+S9) induced significant (≤0.01) increases in the percentage of cells with structural chromosome aberrations.

2. Confirmatory assay/ repeat nonactivated assay: In the nonactivated confirmatory assay, a precipitate was observed after dosing at ≥850 µg/mL. However, due to the lack of availability of a high dose with adequate cytotoxicity for a valid assay, the confirmatory assay in the absence of S9 was repeated with 11 concentrations ranging from 62.5 to 700 µg/mL. MIs were reduced ≥84% at ≥ 500 µg/mL and by 57 and 66 % at 450 and 475 µg/mL, respectively. Accordingly, 450 µg/mL was selected as the starting level for the analysis of metaphases. Summarized data for the repeat trial are presented in Table 3 (Study report Table 7). As shown, significant (≤0.01) increases in the percentage of cells with structural chromosome aberrations (4.5 and 4%, respectively) were scored at 400 and 450 µg/mL, and simple breaks predominated; however, a chromatid exchange was scored in the high-dose group

In the S9-activated confirmatory assay, a precipitate was observed after dosing, at wash, and at harvest at >925 µg/mL. The high dose selected for analysis, 1000 µg/mL, had a 55% reduction in MI compared to the vehicle control (Table 4; Study report Table 9). Significant ($p \leq 0.01$) increases were recorded at 800 and 1000 µg/mL 4.0 and 8%, respectively. Simple breaks were the major type of structural chromosome aberration (4% at both levels); however, 2% of the scored cells at the high concentration had chromatid exchanges. The remaining concentrations were negative for structural chromosomal aberrations, polyploidy, and endoreduplication. The study author argued that the significant effect observed at 800 µg/mL, (4% of scored cells with structural chromosomal aberrations, was judged to be most likely a statistical anomaly due to the low incidence of chromosomal aberrations (0%) in the vehicle control cultures compared to historical vehicle control values (mean 0.4±0.53, N =

87). In addition, the results were negative for inducing chromosomal aberrations at 750 µg/mL, 850 µg/mL, and 925 µg/mL. Therefore, the slight increase in chromosomal aberrations observed at 800 µg/mL was considered by the study author not be related to treatment with the test material. Similarly, the study author argued that the single high dose culture analyzed at 1000 µg/mL had a 55% reduction in the MI compared to the vehicle control cultures, raising the possibility of cytotoxicity inducing clastogenicity, and especially in this instance when it also was a precipitating concentration.

The sensitivity of the test system to detect the induction of chromosomal aberrations in both the absence and presence of S9 was shown by the significant ($p \leq 0.01$) increases in the percentage of cells with structural chromosome aberrations in cultures exposed to the positive control agents.

Table 1: In vitro cytogenetics initial assay with MON 52708: Results in the absence of S9

Test material[a] (µg/mL)	# cells scored for pp and er[b]	% mitotic index reduction	# of pp cells[b]	# of er cells[b]	Number of cells showing structural chromosome aberrations by type of aberration					
					Gaps	Breaks	Chte[b]	Chre[b]	MAB[b]	Totals[c] -g
RPMI 1640	200	--	0	0	3	0	0	0	0	3
DMSO 1%	200	0	0	0	1	2	0	0	0	2
MMC 0.3	100	--	0	0	7	29	13	0	0	39 %**
MON 52708										
420	200	0	0	0	3	0	0	0	0	0
600	200	13	1	1	1	1	0	0	0	1 0.5 %
858	200	38	1	2	0	2	0	0	0	2 1.0 %
1230	200[d]	57	0	0	1	4	2	0	1	6 3.0 %

[a] Test materials – RPMI 1640 = negative control culture medium; DMSO = vehicle control (dimethyl sulfoxide); MMC = positive control (mitomycin C).
[b] pp = polyploidy; er = endoreduplication; Chte = chromatid exchange; Chre = chromosome exchange; MAB = multiple aberrations, >4.
[c] –g = number of cells with chromosome aberrations, -gaps; values in % = percentage of cells with structural chromosome aberrations.
[d] Due to excessive cytotoxicity, all cells scored came from a single culture rather than duplicate cultures.
** = Significantly ($p \leq 0.01$) increased (-g).
Source: Study report Table 2, p.28 (MRID 47899510).

Table 2: In vitro cytogenetics initial assay with MOM 52708: Results in the presence of S9

Test material[a] (µg/mL)	# cells scored for pp and er[b]	% mitotic index reduction	# of pp cells[b]	# of er cells[b]	Number of cells showing structural chromosome aberrations by type of aberration					
					Gaps	Breaks	Chte[b]	Chre[b]	MAB[b]	Totals[c] -g
RPMI 1640	200	--	2	0	2	1	0	0	0	1
DMSO 1%	200	0	1	0	2	0	0	0	0	0
CP 25.0	100	--	0	0	6	30	9	0	0	35 %**
MON 52708										
294	200	0	1	0	1	1	0	0	0	1 0.5%
420	200	15	2	1	3	1	0	0	0	1 0.5%
600	200	33	0	0	3	5	1	0	0	5 2.5%
858	200	56	0	1	1	13 6.5%	4 2%	0	3	19** 9.5%

[a] Test materials – RPMI56 1640 = negative control culture medium;
DMSO = vehicle control (dimethyl sulfoxide);
CP = positive control (cyclophosphamide)
[b] pp = polyploidy; er = endoreduplication; Chte = chromatid exchange; Chre = chromosome exchange; MAB = multiple aberrations, >4.
[c] –g = number of cells with chromosome aberrations, -gaps; values in % = percentage of cells with structural chromosome aberrations.
** = Significantly increased at $p \leq 0.01$ (-g).
Source: Study report Table 4, p. 30 (MRID 47899510).

Table 3: In vitro cytogenetics confirmatory assay with DCSA: Results in the absence of S9 (REPEAT)

Test material[a] µg/mL	# cells scored for pp and er[b]	% mitotic index reduction	# of pp cells[b]	# of er cells[b]	Number of cells showing structural chromosome aberrations by type of aberration					
					Gaps	Breaks	Chte[b]	Chre[b]	MAB[b]	Totals[c] -g
RPMI 1640	200	--	0	0	1	0	0	0	0	0
DMSO 1%	200	0	0	0	2	0	0	0	0	0
MMC 0.3	100	--	0	0	8	26	21	0	0	40%**
MON 52708										
250	200	14	0	0	3	2	0	0	0	2 1.0%
350	200	37	0	0	4	5	0	0	0	5 2.5 %
400	200	31	0	0	9	8 4.0%	0	0	0	8** 4.0%
450	200	57	0	0	11	9 4.5%	1 0.5%	0	0	9** 4.5%

[a] Test materials – RPMI 1640 = negative control culture medium; DMSO = vehicle control (dimethyl sulfoxide); MMC = positive control (mitomycin C).
[b] pp = polyploidy; er = endoreduplication; Chte = chromatid exchange; Chre = chromosome exchange; MAB = multiple aberrations, >4.
[c] –g = number of cells with chromosome aberrations, -gaps ; values in % = percentage of cells with structural chromosome aberrations.
** = Significantly increased at $p \leq 0.01$ (-g).

Source: Study report Table 7, p. 33 (MRID 47899510).

Table 4: In vitro cytogenetics confirmatory assay with MON 52708: Results in the presence of S9

Test material[a] µg/mL	# cells scored for pp and er[b]	% mitotic index reduction	# of pp cells[b]	# of er cells[b]	Number of cells showing structural chromosome aberrations by type of aberration					
					Gaps	Breaks	Chte[b]	Chre[b]	MAB[b]	Totals[c] -g
RPMI 1640	200	--	0	0	0	0	0	0	0	0
DMSO 1%	200	0	0	0	0	0	0	0	0	0
CP 25.0	125	--	0	0	3	32	5	0	1	36 %
MON 52708										
750	200	0	0	0	0	3	2	0	0	5 2.5%
800	200	0	0	0	6	8	1	0	0	8** 4.0%
850	200	18	0	0	0	2	3	0	0	4 2.0%
925	200	38	0	0	3	4	0	0	0	4 2.0%
1000	200	55	1	0	0	4 2%	4 2%	0	1	16** 8.0%

[a] Test materials – RPMI 1640 = negative control culture medium; DMSO = vehicle control (dimethyl sulfoxide); CP = positive control (cyclophosphamide); all others are DCSA concentrations.
[b] pp = polyploidy; er = endoreduplication; Chte = chromatid exchange; Chre = chromosome exchange; MAB = multiple aberrations, >4.
[c] –g = number of cells with chromosome aberrations, -gaps; values in % = percentage of cells with structural chromosome aberrations.
** = Significantly ($p \leq 0.01$); (-g).
Source: Study report Table 9, p. 33 (MRID 47899510).

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:
Reliability Rating: Unacceptable Not Reliable
This study is compliant with OECD 449(1997)

C. CONCLUSIONS:

MON 52708 was considered negative by the study authors in the absence of S9 and weakly positive for inducing chromosomal aberrations in cultured human peripheral blood lymphocytes in the presence of S9 at a single toxic, high dose," the biological relevance of which is very debatable". Although the study author dismissed the positive findings in the S9-activated confirmatory trial as either anomalous or indirectly associated with cytotoxicity, we disagree and believe that no conclusions can be reached. The lack of agreement between the MI results of the initial and confirmatory S9-activated assays, the testing of precipitating concentrations, the occurrence of significant results, while at different concentrations for both trials of the S9-activated tests and in 1 of 2 trial without S9, and the occurrence of complex aberrations in both S9-activated trail and in the repeat nonactivated trial render these data inconclusive.

Revised by U.S. Environmental Protection Agency

In vitro Mammalian Chromosome Aberration Test

Report: IIA 5.4.2/01	Murli, H. (2007). Chromosomal Aberrations in cultured human peripheral blood lymphocytes with MON 11900. Covance Laboratories Inc., Vienna, Va. Monsanto report no. CV-2006-083; April 19, 2010; unpublished. MRID NO. 47899527 .
Dates of work:	August 21, 2006 October 26, 2006
Guidelines:	OPPTS 870. 5375 (August, 1998) OECD 473 (July 21, 1997) MAFF 12- Shirasu, 1988
GLP: Yes	OECD Principles of GLP, ENV/MC/CHEM (1998) Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.
GLP: Exceptions	No

Executive Summary:

In an in vitro chromosome aberration test (MRID 47899527), cultured human lymphocytes were exposed to MON 11900 (Purity 94.6%, Lot/Batch No. GLP-0604-17184-T) prepared in dimethyl sulfoxide (DMSO), in independent experiments and were evaluated for clastogenic potential. On the basis of the assessment of reduced mitotic indices (MIs), the initial assay treatment period was 3 hours with a 22-hour harvest and was conducted with 0, 16.3, 23.3, 33.2, 47.5, 67.8, 96.8, 138, 198, 282, 403, 576, 823, 1180, 1680, and 2400 ug/mL +/-S9. Cultures treated with 0, 576, 823, 1180, and 1680 ug/mL +/-S9 analyzed for chromosome aberrations. The highest dose tested was in excess of 10 mM.

In the confirmatory chromosomal assay, the treatment period was 22 hours without S9 and 3 hours with S9; cells were harvest at the end of the nonactivated exposure or 22 hours after the S9-activated exposure. The range of test concentrations was 113 to 2400 ug/mL -S9 and 450 to 2400 ug/mL +S9; cells exposed to 225, 338, 675, and 900 ug/mL -S9 and 450, 900, 1600, and 2400 ug/mL +S9 were scored for chromosome aberrations. The S9 homogenate was derived from the liver of rats induced with Aroclor 1254. Mitomycin C and cyclophosphamide served as the positive controls.

MON 11900 was tested up to adequately high concentration, producing ~ 55% reduction in the MI after 3 hours of treatment and a 22- hour harvest (Initial trial: 1680 µg/mL +/–S9) or a 54% reduction with a prolonged 22-hour treatment (Confirmatory trial: 900 µg/mL –S9) or a 48% reduction with a 3-hour treatment and a 22- hour harvest and a slightly higher percent of S9 in the S9 cofactor mix (Confirmatory trial 2400 µg/kg +S9). However, the test material precipitated at concentrations ≥1200 µg/mL +/-S9 . While the initial trial was negative, significant increases ($p \leq 0.01$) in cells with structural chromosome aberrations were seen at 225, 675 and 900 µg/mL-S9 (5.0, 16.0 and 16.0 % cells with aberrations versus 0.5 % in the solvent control group) and at

2400 µg/kg +S9 (30.0 % cells with aberrations versus 0.5 % in the solvent control group). For the nonactivated test concentrations, the response was dose-related and the predominant type of chromosome aberration was simple breaks. With S9, the response was limited to the high dose and the predominant type of chromosome aberration was simple breaks; however, chromatid exchanges, which are complex aberrations, were also seen.

The study author concluded that the increase in structural chromosomes at the limit concentration of MON 11900 in the presence of S9 activation has "debatable biological relevance". While it was only seen at a precipitating level, the increase was both significant and substantial (30% of the cells examined had structural chromosome aberrations versus 0.5 % in the solvent control cultures) and accompanied by an 8% increase in cells with chromatid exchanges, a complex aberration that is not frequently scored.

Based on these considerations, we conclude that the S9-activated portion of the assay should have been repeated. Until this issue is resolved, it is concluded that MON 11900 was positive in this test system at 675 and 900 µg/mL –S9 but only after the prolonged exposure 22 hours and positive with S9 at 2400 µg/mL.

This study is classified as totally reliable (acceptable/guideline) and satisfies the guideline requirement for *in vitro* mammalian cytogenetics (chromosome aberrations) OCSPP 870.5375; OECD 473.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	MON 11900
Description:	White solid
Lot/Batch#:	GLP-0604-17184-T
Purity:	94.6%
CAS #:	None provided
Stability of test compound:	Expiration date listed as April 26, 2007.

2. Control materials

Negative control:	Culture Medium (RPMI 1640)
Solvent control:	DMSO /1%
Positive control:	Non-activation: Mitomycin C (MMC) (0.75, 1.0, and 1.5 µg/mL, 3-hour treatment; 0.2, 0.3, and 0.4 µg/mL, ~22-hour treatment) Activation: Cyclophosphamide (CP, 200, 25.0, and 40.0 µg/mL) Both MMC and CP were dissolved in sterile, deionized water

3. Activation: S9 derived from 7-week old, male Sprague-Dawley rats (206-247 g)

X	Induced	X	Aroclor 1254	X	Rat	X	Liver
	Non-induced		Phenobarbital		Mouse		Lung
			β-naphthoflavone		Hamster		Other
			Other		Other		

The S9 fraction was obtained from (Molecular Toxicology, Inc. Aliquots of S9 were thawed immediately before use and added to the other components to form the activation system. As described below, two separate S9 mixes were used:

S9 Activation System (Initial Chromosome Aberration Assay)

Component	**Concentration in Cultures**
NADP (sodium salt)	1.5 mg/mL (1.8 mM)
Isocitric acid	2.7 mg/mL (10.5 mM)
Homogenate (S9 fraction)	15.0 µL/mL (1.5%)

S9 Activation System (Confirmatory Chromosome Aberration Assay)

COMPONENT	**CONCENTRATION IN CULTURES**
NADP (sodium salt)	0.66 mg/mL (0.84 mM)
Glucose-6-Phosphate	0.27 mg/mL (1.05 mM)
$MgCl_2$ ($6H_2O$)	0.10 mg/mL (1.05 mM)
KCl	0.52 mg/mL (6.9 mM)
HEPES	0.20 mg/mL (0.84 mM)
Homogenate (S9 fraction)	18.0 µL/mL (1.8%)

4. **Test cells:** Mammalian cells in culture

[ ]	Mouse lymphoma L5178Y cells	[ ]	V79 cells (Chinese hamster lung fibroblasts)
[ ]	Chinese hamster ovary (CHO) cells	[ ]	list any others
[ ]	Human peripheral lymphocytes	[X]	Human whole blood lymphocytes.

Properly maintained?	[X]	Yes		No
Periodically checked for Mycoplasma contamination?	[ ]	Yes	Not reported	No
Periodically checked for karyotype stability?	[ ]	Yes	Not reported	No

Media: RPMI 1640 supplemented with HEPES buffer, approximately 20% heat-inactivated fetal bovine serum, penicillin (100 units/mL), streptomycin (100 µg/mL), L-glutamine (2 mM) and 2% phytohemagglutinin M

5. Test compound concentrations used:

c. Preliminary cytotoxicity : Conducted in parallel with the cytogenetic assay.
d. Cytogenetic assay: Duplicate cultures for each test dose, vehicle or positive control

Initial Trial:

Concentrations of MON 11900 tested (µg/mL)

3 hours, without S9	0	16.3	23.3	33.2	47.5	67.8	96.8	138	198	282	403	**576**	**823**	**1180**	**1680**	2400
3 hours, with S9	0	16.3	23.3	33.2	47.5	67.8	96.8	138	198	282	403	**576**	**823**	**1230**	**1680**	2400

IIIA 10.1.4.1 Confirmatory Trial

Concentrations of MON 11900 tested (µg/mL)

22 hours, without S9	0	113	**225**	**338**	450	**675**	**900**	1200	1600	2000	2400
3 hours, with S9	0	**450**	**900**	1200	**1600**	2000	**2400**				

Concentrations bolded were examined for chromosomal aberrations.

IIIA 10.1.5 B. STUDY DESIGN AND METHODS

1. In life dates: August 21, 2006—October 26, 2006

2. Treatment:

Preliminary cytotoxicity assay: Cytotoxicity was determined in parallel with the cytogenetic assays by determining the mitotic index (MI) by counting the number of mitosis in 1000 cells.
Cytogenetic assay: Independently performed experiments were conducted.

a. Cell exposure time:

	Test material	Solvent control	Positive control
Non-activated:	3 h 22 h	3 h 22 h	3 h 22 h
Activated:	3 h	3 h	3 h

b. Spindle inhibition:

Inhibition used/concentration:	Colcemid/ 0.1 μg/mL
Administration time:	2 hours (before cell harvest)

c. Cell harvest time after termination of treatment:

	Test material	Solvent control	Positive control
Non-activated and Activated:	22, 0 & 22 h	22, 0 & 22 h	22, 0 & 22 h

d. Details of slide preparation: Following the colchicine treatment, cells were swollen with 75 mM KCl, fixed with a 3:1mixture of methanol and glacial acetic acid, and stained with 5% Giemsa. Two slides per group per experiment were prepared.

e. Metaphase analysis

No. of cells examined per dose: 200 (100 per duplicate culture in the treatment groups and the negative control. Only one culture from one of the concentrations of the positive control groups was scored.

Scored for structural?	x	Yes		No
Scored for numerical?	x	Yes, polyploidy cells and endoreduplication		No
Coded prior to analysis?	x	Yes		No

d. Evaluation criteria:

Assay validity: The assay is considered acceptable by the testing laboratory if (a) the negative and vehicle control cultures had >5% of the cells with aberrations and the positive control induced a significant (≤0.01) increase in the number of chromosome aberrations.

Positive response: A test substance is concluded to induce a positive response if: the number of cells with structural chromosome aberrations was significantly (≤0.01) increase at one or more concentrations and the increase was dose-related.

g. Statistical analysis: The data were analysed for statistical significance using the Cochran-Armitage test for linear trend and Fisher's exact test at $p \leq 0.01$.

10.1.5.1.1 II. RESULTS AND DISCUSSION

D. Analytical determinations: Analytical determinations were not performed. The test material was insoluble at concentrations ≥2500 µg/mL.

E. Preliminary cytotoxicity test: An independent cytotoxicity test was not performed; cytotoxicity was measured in parallel with the cytogenetic assays.

F. Cytogenetic assays:

a. **Initial assay:** In the initial assay, compound precipitate was noted at ≥1680 µg/mL +/-S9. A 70 and 77% reduction in the MI was seen at 2400 µg/mL +/-S9. At 1680 µg/mL +/-S9, MIs were reduced by 55 %; accordingly, this concentration was selected for the analysis of metaphases. Data from the initial nonactivated and S9-activated trials are presented in Tables 1 and 2 (Study Report Tables 2 and 4, respectively). In the absence or presence of S9 activation, no significant increases in cells with structural or numerical chromosome aberrations were seen at any analysed concentrations. By contrast, the nonactivated and S9-activated positive controls induced the expected significant ($p \leq 0.01$) increase in structural chromosome aberrations.

b. **Confirmatory assay:** Concentrations evaluate in the confirmatory trial ranged from
113 to 2400 µg/mL -S9 and 450 to 2400 µg/mL +S9. Compound precipitation was seen at ≥1200 µg/mL +/-S9. MIs were reduced by ≥69% at ≥1200 µg/mL -S9 and by 48% at 2400 µg/mL +-S9. Based on theses data, 900 µg/mL -S9 (with a reduced MI of 54%) and 2400 µg/mL +S9 were selected for the analysis of metaphases. Data from the confirmatory nonactivated and S9-activated trials are presented in Tables 3 and 4 (Study Report Tables 6 and 8, respectively). In the absence of S9 activation, significant ($p \leq 0.01$) increases in cells with structural chromosome aberrations were seen at 225, 675 and 900 µg/mL (5.0, 16.0 and 16.0 % cells with aberrations versus 0.5 % in the solvent control group). For these test concentrations, the predominant type of chromosome aberration was simple breaks.

With S9 activation, a significant ($p \leq 0.01$) increase in cells with structural chromosome aberrations was seen only at the high insoluble concentration of 2400 µg/mL (30% cells with aberrations versus 0.5 % in the solvent control group). The predominant type of chromosome aberration was simple breaks (22%); however, 8% of the scored cells had complex aberrations (chromatid exchanges). No significant increases in cells with structural or numerical chromosome aberrations were seen at any the remaining concentrations. The nonactivated and S9-activated positive controls induced the expected significant ($p \leq 0.01$) increase in structural chromosome aberrations.

IIIA 10.1.6 Table 1: Chromosomal Aberrations in Human Lymphocytes - Without Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest—Initial Trial

Assay No.: 28354-0-449OECD Trial No.: B1 Date: 08/31/06 Lab No.: CY083106A Test Article: MON 11900

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judgement (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations: gaps	simple breaks	chte	chre	mab	Totals[c]: -g	Totals[c]: +g	Judgement (+/-)[d]
Controls																	
Negative:	RPMI 1640		A	100		100	1	0		2					0	2	
			B	100		100	0	0		4					0	4	
			Total	200		200				6					0	6	
			Average	%	--		0.5	0.0		3.0					0.0	3.0	
Vehicle:	DMSO	10.0 µL/mL	A	100		100	1	0		7	1				1	8	
			B	100		100	0	0		4	1				1	5	
			Total	200		200				11	2				2	13	
			Average	%	0		0.5	0.0		5.5	1.0				1.0	6.5	
Positive:	MMC	1.00 µg/mL	A	50		100	0	0		7	11	18			24	27	
			B	64		100	0	0		14	13	13	1		25	32	
			Total	114		200				21	24	31	1		49	59	
			Average	%	--		0.0	0.0	-	10.4	21.1	27.2	0.9		43.0	51.8	+
Test Article		576 µg/mL	A	100		100	1	0		5					0	5	
			B	100		100	0	0		3					0	3	
			Total	200		200				8					0	8	
			Average	%	0		0.5	0.0	-	4.0					0.0	4.0	-
		823 µg/mL	A	100		100	0	0		2	1				1	3	
			B	100		100	0	0		4					0	4	
			Total	200		200				6	1				1	7	
			Average	%	26		0.0	0.0	-	3.0	0.5				0.5	3.5	-
		1180 µg/mL	A	100		100	0	0		3					0	3	
			B	100		100	0	0		3					0	3	
			Total	200		200				6					0	6	
			Average	%	45		0.0	0.0	-	3.0					0.0	3.0	-
		1680 µg/mL	A	100		100	0	0		4					0	4	
			B	100		100	0	0		6					0	6	
			Total	200		200				10					0	10	
			Average	%	55		0.0	0.0	-	5.0					0.0	5.0	-

chte: chromatid exchange chre: chromosome exchange
mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication
[a] % Mitotic index reduction as compared to the vehicle control.
[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.
[c] -g = # or % of cells with chromosome aberrations +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.
[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. RPMI 1640 = culture medium
DMSO = dimethylsulfoxide
MMC = Mitomycin C
Source: Study Report, Table 2, p. 22 (MRID 47899527).

Table 2: Chromosomal Aberrations in Human Lymphocytes - With Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest—Initial Trial

Assay No.: 28354-0-449OECD Trial No.: B1 Date: 08/31/06 Lab No.: CY083106A Test Article: MON 11900

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and/or er	# of pp Cells	# of er Cells	Judgement (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations: Single gaps	Single breaks	chte	chre	mab	Totals[c] -g	Totals[c] +g	Judgement (+/-)[d]
Controls																	
Negative:	RPMI 1640		A	100		100	0	0							0	0	
			B	100		100	0	0		3	1				1	4	
			Total	200		200				3	1				1	4	
			Average	%	--		0.0	0.0		1.5	0.5				0.5	2.0	
Vehicle:	DMSO	10.0 µL/mL	A	100		100	0	0		2	2				2	4	
			B	100		100	0	0		2					0	2	
			Total	200		200				4	2				2	6	
			Average	%	0		0.0	0.0		2.0	1.0				1.0	3.0	
Positive:	CP	25.0 µg/mL	A	25		100	0	0		5	5	2			7	8	
			B	25		100	0	0			8	1			8	8	
			Total	50		200				5	13	3			15	16	
			Average	%	--		0.0	0.0	-	2.0	26.0	6.0			30.0	32.0	+
Test Article		576 µg/mL	A	100		100	0	0		2					0	2	
			B	100		100	0	0		1					0	1	
			Total	200		200				3					0	3	
			Average	%	7		0.0	0.0	-	1.5					0.0	1.5	-
		823 µg/mL	A	100		100	0	0			1				1	1	
			B	100		100	0	0		1					0	1	
			Total	200		200				1	1				1	2	
			Average	%	33		0.0	0.0	-	0.5	0.5				0.5	1.0	-
		1180 µg/mL	A	100		100	0	0		1	1				1	2	
			B	100		100	0	0		3					0	3	
			Total	200		200				4	1				1	5	
			Average	%	43		0.0	0.0	-	2.0	0.5				0.5	2.5	-
		1630 µg/mL	A	100		100	0	0		2					0	2	
			B	100		100	0	0		1	1				1	2	
			Total	200		200				3	1				1	4	
			Average	%	55		0.0	0.0	-	1.5	0.5				0.5	2.0	-

chte: chromatid exchange chre: chromosome exchange
mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication
a % Mitotic index reduction as compared to the vehicle control.
[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.
[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.
[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. RPMI 1640 = culture medium
DMSO = dimethylsulfoxide
CP = Cyclophosphamide
Source: Study Report, Table 4, p. 24 (MRID 47899527).

Table 3: Chromosomal Aberrations in Human Lymphocytes - Without Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest—Confirmatory Trial

Assay No.: 28354-0-449OECD Trial No.: C1 Date: 09/27/06 Lab No.: CY092606 Test Article: MON 11900

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judgement (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations							Judgement (+/-)[d]
										gaps	simple breaks	chte	chre	mab	Totals[c] -g	Totals[c] +g	
Controls																	
Negative:	RPMI 1640		A	100		100	0	0		3	1				1	4	
			B	100		100	0	0		5	1				1	6	
			Total	200		200				8	2				2	10	
			Average	%	--		0.0	0.0		4.0	1.0				1.0	5.0	
Vehicle.	DMSO	10.0 µL/mL	A	100		100	0	0		5					0	5	
			B	100		100	0	0		3	1				1	4	
			Total	200		200				8	1				1	9	
			Average	%	0		0.0	0.0		4.0	0.5				0.5	4.5	
Positive:	MMC	0.300 µg/mL	A	50		100	0	0		2	13	6		4	22	22	
			B	50		100	0	0		5	11	5		3	19	22	
			Total	100		200				7	24	11		7	41	44	
			Average	%	--		0.0	0.0	-	7.0	24.0	11.0		7.0	41.0	44.0	+
Test Article		225 µg/mL	A	100		100	0	0		1	5	1			6	7	
			B	100		100	0	0		4	4				4	8	
			Total	200		200				5	9	1			10	15	
			Average	%	2		0.0	0.0	-	2.5	4.5	0.5			5.0	7.5	+
		338 µg/mL	A	100		100	0	0			3				3	3	
			B	100		100	0	0			2				2	2	
			Total	200		200					5				5	5	
			Average	%	24		0.0	0.0	-		1.5				1.5	1.5	-
		675 µg/mL	A	75		100	0	0		1	12				12	13	
			B	75		100	0	0		4	12				12	15	
			Total	150		200				5	24				24	28	
			Average	%	37		0.0	0.0	-	3.3	16.0				16.0	18.7	+
		900 µg/mL	A	75		100	0	0		1	13	1			13	14	
			B	75		100	0	0		1	10	1			11	12	
			Total	150		200				2	23	2			24	26	
			Average	%	54		0.0	0.0	-	1.3	15.3	1.3			16.0	17.3	+

chte: chromatid exchange chre: chromosome exchange
mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication
[a] % Mitotic index reduction as compared to the vehicle control.
[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.
[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.
[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. RPMI 1640 = culture medium
DMSO = dimethyl sulfoxide
MMC = Mitomycin C
Source: Study Report, Table 6, p. 26 (MRID 47899527).

Table 4: Chromosomal Aberrations in Human Lymphocytes - With Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest—Confirmatory Trial

Assay No.: 28354-0-449OECD Trial No.: C1 Date: 09/27/06 Lab No.: CY092606 Test Article: MON 11900

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judgement (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations: single gaps	single breaks	chte	chre	mab	Totals[c] -g	Totals[c] +g	Judgement (+/-)[d]
Controls:																	
Negative:	RPMI 1640		A	100		100	0	0		2	1				1	3	
			B	100		100	0	0			1				1	1	
			Total	200		200				2	2				2	4	
			Average	%	–		0.0	0.0		1.0	1.0				1.0	2.0	
Vehicle:	DMSO	10.0 µL/mL	A	100		100	0	0			1				1	1	
			B	100		100	0	0		2					0	2	
			Total	200		200				2	1				1	3	
			Average	%	0		0.0	0.0		1.0	0.5				0.5	1.5	
Positive:	CP	40.0 µg/mL	A	50		100	0	0		6	9	4		4	16	21	
			B	50		100	0	0		7	22	2		5	28	33	
			Total	100		200				13	31	6		9	44	54	
			Average	%	–		0.0	0.0	-	13.0	31.0	6.0		9.0	44.0	54.0	+
Test Article		450 µg/mL	A	100		100	1	0		1					0	1	
			B	100		100	0	0		1	1				1	2	
			Total	200		200				2	1				1	3	
			Average	%	5		0.5	0.0	-	1.0	0.5				0.5	1.5	-
		900 µg/mL	A	100		100	0	0		1	1				1	2	
			B	100		100	0	0		1	2	1			3	4	
			Total	200		200				2	3	1			4	6	
			Average	%	10		0.0	0.0	-	1.0	1.5	0.5			2.0	3.0	-
		1800 µg/mL	A	100		100	0	0		2					0	2	
			B	100		100	0	0		1	2				2	3	
			Total	200		200				3	2				2	5	
			Average	%	30		0.0	0.0	-	1.5	1.0				1.0	2.5	-
		2400 µg/mL	A	50		100	0	0		3	12	3		1	16	18	
			B	50		100	0	0		2	10	5		2	14	15	
			Total	100		200				5	22	8		3	30	33	
			Average	%	48		0.0	0.0	-	5.0	22.0	8.0		3.0	30.0	33.0	+

chte: chromatid exchange chre: chromosome exchange
mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. RPMI 1640 = culture medium
DMSO = dimethylsulfoxide
CP = Cyclophosphamide

Source: Study Report, Table 8, p. 28 (MRID 47899527).

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:

RELIABILITY RATING: reliable
This study is fully compliant with OECD 473 (1997)

G. CONCLUSIONS: MON 11900 was tested up to adequately high concentration, producing ~ 55% reduction in the MI after 3 hours of treatment and a 22- hour harvest (Initial trial: 1680 μg/mL +/–S9) or a 54% reduction with a prolonged 22-hour treatment (Confirmatory trial: 900 μg/kg –S9) or a 48% reduction with a 3-hour treatment and a 22-hour harvest and a slightly higher percent of S9 in the S9 cofactor mix (Confirmatory trial 2400 μg/kg +S9). However, the test material precipitated at this concentration. While the initial trial was negative, significant increases ($p \leq 0.01$) in cells with structural chromosome aberrations were seen at 225, 675 and 900 μg/mL- S9 (5.0, 16.0 and 16.0 % cells with aberrations versus 0.5 % in the solvent control group) and at 2400 μg/kg +S9 (16.0 % cells with aberrations versus 0.5 % in the solvent control group). For the nonactivated test concentrations, the response was dose-related and the predominant type of chromosome aberration was simple breaks. With S9, the response was limited to the high dose and the predominant type of chromosome aberration was simple breaks; however, chromatid exchanges, which are complex aberrations, were also seen.

These data clearly show that the nonactivated test material was clastogenic but only after the prolonged 22-hour exposure. The study author concluded that the increase in structural chromosomes at the limit concentration of MON 11900 in the S9-activated phase of testing has debatable biological relevance. While it was only seen at a precipitating level, the increase was both significant and substantial (30% of the cells examined had structural chromosome aberrations versus 0.5 % in the solvent control cultures) and accompanied by an 8% increase in cells with chromatid exchanges, a complex aberration that is not frequently scored. **Based on these considerations we assess that this portion of the assay should have been repeated. Until this issue is resolved, it is concluded that MON 11900 was positive in this test system at 675 and 900 μg/mL –S9 but only after the prolonged exposure 22 hours and positive with S9 at 2400 μg/mL.**

Revised by U.S. Environmental Protection Agency

	***In Vivo* Mammalian Cytogenetics – Chromosome aberration Assay in Rats**
Report:	IIA 5.8/11. Xu, Y (2008). Chromosomal aberrations in vivo in rat bone marrow cells with MON 52708; Covance Labs., Vienna, Va.; Report No. CV-2006-141; unpublished, MRID 47899513.
Dates of work:	November 09, 2006 - January 05, 2007
Guidelines:	OECD 475(1997), EPA OPPTS 870.5385 (1998), MAFF (Shirasu, 1988) PMRA DACO 4.5.4
GLP:	Yes
GLP Exceptions:	None

EXECUTIVE SUMMARY:

In an in vivo chromosome aberration test (MRID 47899513), groups of five male and five female CD (SD) IGS BR rats, were exposed once via oral gavage to MON 52708 (Purity 97.9%, Lot/Batch no. GLP-0603-16958-T), prepared in corn oil) at 0, 400, 800, or 1600 mg/kg (males) and 0, 300, 600, or 1200 mg/kg (females). Bone marrow was harvested at 18 hours (all groups) and 42 hours (highest test group and the vehicle group) and examined for structural chromosomal aberration. Cyclophosphamide (CP) at 60 mg/kg was included as the positive control.

Following a toxicity range-finding study using 3/sex/dose, the maximum dose tolerated (MTD) was estimated to be 1500 mg/kg, based on mortality (1 M & 3 F at 2000 mg/kg and 1 F at 1500 mg/kg) and other signs of toxicity.

In the definitive study, one male and one female died at 1600 and 1200 mg/kg, respectively. Other clinical signs at the highest doses tested for both sexes included squinted eyes, hunched posture, and irregular respiration. The test material was, however, not cytotoxic or clastogenic to the target organ in either sex at any dose or harvest time. Controls from corn oil and cyclophosphamide were within the expected normal historical ranges.

Based on these findings, it is concluded that MON 52708 did not cause an increase in the number of chromosome aberrations in rat bone marrow cells at dose levels up to and including 1600 mg/kg by oral gavage in males or 1200 mg/kg in female rats in this study.

This study is classified as **totally reliable (acceptable/guideline)** and satisfies the guideline requirement for in vivo chromosme aberration data (OCSPP 870.5385; OECD 475).

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** DCSA (MON 52708), 3,6-Dichlorosalicylic acid
   Description: White powder
   Lot/Batch#: GLP-0603-16958-T
   Purity: 97.9%
   CAS #: None given
   Stability of test compound: Listed with an expiration date of March 20, 2007.

2. **Control materials**
   Negative control: None
   Solvent control: Corn oil /10 ml/kg
   Positive control: Cyclophosphamide (CP, 60 mg/kg)

3. **Test system:**

Species:	Rat
Strain:	Sprague Dawley CD® (SD)IGS BR
Age:	89 weeks
Weight at dosing:	268 – 311g (M); 201 – 232g (F) (initiation of treatment, main test)
Source:	Charles River Labs., Raleigh, NC
Acclimation period:	5 days
Diet:	PMI Certified Rodent Diet® # 5002, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Five/sex/cage/ with bedding
No. of animals used per dose/time point:	3 Males 3 Females (Dose-range finding) 5 Males 5 Females (Main assay)

4. **Environmental conditions:**

Temperature:	64 – 79°F
Humidity:	30 – 70%
Air changes:	10 or more/hour
Photoperiod:	12 hours light / 12 hours darkness

5. Test compound concentrations used:

	Dose levels	Final volume	Route
Preliminary dose-range finding	1500 and 2000 mg/kg (3 M; 3F)	10 mL/kg	Oral gavage
Main study	0, 400, 800, 1600 mg/kg (5 M) 0, 300, 600, 1200 mg/kg (5 F)	10 mL/kg 10 mL/kg	Oral gavage

B. STUDY DESIGN AND METHODS:

1. Preliminary cytotoxicity assay: Doses for the main study were determined from the results of a toxicity study using only 3 mice per sex per dose at 1500, 2000 mg/kg of the test material in a single gavage dose. The animals were observed for 2 days and mortality and toxic signs were assessed.

2. Micronucleus assay:

Treatment and sampling times

a. Test compound and vehicle control

Dosing:	[X]	Once	[ ]	twice (24 hrs apart)			[ ]	Other		
Sampling (after last dose):	[ ]	6 hr	[ ]	12 hr	[X]	24 hr	[X]	42 hr High dose & vehicle	[ ]	72 hr

b. Positive control

Dosing:	[X]	Once	[ ]	twice (24 hrs apart)			[ ]	Other		
Sampling (after last dose):	[ ]	6 hr	[ ]	12 hr	[X]	18 hr	[ ]	48 hr	[ ]	72 hr

c. Details of slide preparation: At the appropiate harvest internal, groups of animals were sacrificed and bone marrow cells were collected, mixed with Hank's balanced salt solution and centrifuged. Cell pellets were resuspended in hypotonic KCl (0.075 M), fixed with a 3:1mixture of methanol and glacial acetic acid, and stained with 5% Giemsa.

d. Metaphase analysis

No. of cells examined per dose: If available, 100 per animalper sex for vehicle and treatment groups; 25/animal for the positive control were scored.

Scored for structural?	x	Yes		No
Scored for numerical?	x	Yes, polyploidy cells and endoreduplication		No
Coded prior to analysis?	x	Yes		No

Note: The mitotic index (MI) was determined from the count of cells in mitosis per 1000 cells.

e. Evaluation criteria:

Assay validity: The assay was considered valid if the structural aberration frequency was between 0 and the highest values in the historical control data (5.0, M at 12-24 hours; 3.0, M at 42-48 hours) and the positive control group produced a detectable increase over background.

Positive response: The test material was considered positive if it induced a significantly increased and dose-related for at least one dose.

e. Statistical analysis: The data were analyzed for significance using nonparametric ranked analysis techniques at $p \leq 0.05$.

II. RESULTS AND DISCUSSION

A. Analytical determinations: Analytical determinations were not conducted.

B. Preliminary dose-range finding test: In the initial range-finding study, all female rats succumbed to treatment in the 2000-mg/kg group and 1 of 3 males also died at this dose. One female died at 1500 mg/kg. Other signs of clinical toxicity included hunched posture, hypoactivity, and piloerection (both sexes) at 2000 mg/kg. Based on these findings, the high dose for the definitive study was 1600 mg/kg (males) and 1200 mg/kg (females).

C. Cytogenetic assay: One male and one female died at 1600 and 1200 mg/kg, respectively. Other clinical signs at the highest doses tested for both sexes included squinted eyes, hunched posture, and irregular respiration.

Data for chromosomal aberrations are summarized on Tables 1 (males) and Table 2 (females) (Study Report Tables 6 and 7, respectively). As shown, the MIs, were unaffected by treatment. Similarly, no significant increases in the percentage of cells with structural or numerical aberrations were seen in any treatment group at the 18- or 42- hour harvests. By contrast, CP induced a significant decrease in the MIs for both

sexes and caused marked and significant (≤0.05) increases in the frequency of chromosome aberrations in both sexes.

Table 1: Chromosome Aberrations in Rat Bone Marrow – Summary – Males

Assay No.: 28298-0-452OECD Lab No.: CY112306
Test Article: MON 52708 Initiation of Dosing: 11/28/06

Treatment	Dose Level	Harvest Time (~ hr after last dose)	Number of Animals	Total Number of Cells Analyzed for Aberrations	% -g Group Mean ± S.E.	% +g Group Mean ± S.E.	Judgment (+/-)[a]	% Polyploidy Group Mean ± S.E.	% Endoreduplication Group Mean ± S.E.	Judgment (+/-)[b]	% Mitotic Index Group Mean ± S.E.
Controls											
Corn Oil	10 mL/kg	18	5	500	0.0 ± 0.00	0.0 ± 0.00		0.2 ± 0.20	0.0 ± 0.00		10.8 ± 0.68
		42	5	500	1.0 ± 0.55	3.0 ± 1.05		0.2 ± 0.20	0.0 ± 0.00		4.4 ± 1.90
Cyclophosphamide	60 mg/kg	18	5	125	67.2 ± 8.52	69.6 ± 7.44	+	0.2 ± 0.20	0.0 ± 0.00	-	1.7 ± 0.38[c]
Test Article											
	400 mg/kg	18	5	500	0.0 ± 0.00	1.6 ± 0.93	-	0.2 ± 0.20	0.0 ± 0.00	-	6.9 ± 2.14
	800 mg/kg	18	5	500	0.0 ± 0.00	0.6 ± 0.60	-	0.4 ± 0.24	0.0 ± 0.00	-	7.1 ± 2.01
	1600 mg/kg	18	5	500	0.0 ± 0.00	0.4 ± 0.40	-	0.0 ± 0.00	0.0 ± 0.00	-	7.3 ± 0.79[c]
		42	4	400	0.3 ± 0.25	2.5 ± 0.96	-	0.3 ± 0.25	0.0 ± 0.00	-	3.4 ± 0.25

% -g = % of cells with chromosome aberrations.
% +g = % of cells with chromosome aberrations + % of cells with gaps.
a Significantly greater in -g than the corresponding vehicle control, $p \leq 0.05$.
b Significantly greater in polyploidy and endoreduplication than the corresponding vehicle control control, $p \leq 0.05$.
c Significantly less in % mitotic index than the corresponding vehicle control, $p \leq 0.05$.
Source: Study Report Table 6 p 28 (MRID 47899513)

Table 2. Chromosome Aberrations in Rat Bone Marrow – Summary – Females

Assay No.: 28298-0-452OECD Lab No.: CY112306
Test Article: MON 52708 Initiation of Dosing: 11/28/06

Treatment	Dose Level	Harvest Time (~ hr after last dose)	Number of Animals	Total Number of Cells Analyzed for Aberrations	% -g Group Mean ± S.E.	% +g Group Mean ± S.E.	Judgment (+/-)[a]	% Polyploidy Group Mean ± S.E.	% Endoreduplication Group Mean ± S.E.	Judgment (+/-)[b]	% Mitotic Index Group Mean ± S.E.
Controls											
Corn Oil	10 mL/kg	18	5	500	0.6 ± 0.40	1.6 ± 0.98		0.2 ± 0.20	0.8 ± 0.80		7.6 ± 2.07
		42	5	500	0.2 ± 0.20	1.0 ± 1.00		0.0 ± 0.00	0.0 ± 0.00		9.2 ± 2.01
Cyclophosphamide	60 mg/kg	18	5	125	81.6 ± 2.71	84.8 ± 2.65	+	0.0 ± 0.00	0.0 ± 0.00	-	0.6 ± 0.17[c]
Test Article											
	300 mg/kg	18	5	500	0.4 ± 0.40	1.6 ± 0.75	-	0.2 ± 0.20	0.0 ± 0.00	-	3.9 ± 1.32
	600 mg/kg	18	5	500	0.4 ± 0.24	1.4 ± 0.75	-	0.0 ± 0.00	0.0 ± 0.00	-	2.7 ± 1.37
	1200 mg/kg	18	5	500	0.4 ± 0.24	1.6 ± 0.81	-	0.2 ± 0.20	0.0 ± 0.00	-	4.0 ± 0.79
		42	4	400	0.5 ± 0.29	2.3 ± 1.03	-	0.0 ± 0.00	0.0 ± 0.00	-	4.7 ± 2.27

% -g = % of cells with chromosome aberrations.
% +g = % of cells with chromosome aberrations + % of cells with gaps.
a Significantly greater in -g than the corresponding vehicle control, $p \leq 0.05$.
b Significantly greater in polyploidy and endoreduplication than the corresponding vehicle control, $p \leq 0.05$.
c Significantly less in % mitotic index than the corresponding vehicle control, $p \leq 0.05$.
Source: Study Report Table 7 p 29 (MRID 47899513)

II. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:
RELIABILITY RATING: Totally reliable

This study is compliant with OECD 474 (1997)

C. CONCLUSIONS: MON 52708 was tested up to an adequate high dose (1600 mg/kg, M and 1200 mg/kg, F) producing death and other clinical signs but was not cytotoxic to the target organ and failed to induce a clastogenic response at any dose or sacrifice time in either sex. The positive control, CP at 60 mg/kg produced a significant decrease in the MIs and a significant increase in structural chromosme aberraations ($p \leq 0.05$),

Accordingly, the study was negative and well-done; it is acceptable and satisfies OECD 475 and USEPA 870.5385 for an *in vivo* chromome aberration assay.

Deficiencies: None

Revised by U.S. Environmental Protection Agency

In Vivo Mammalian Cytogenetics – Chromosome aberration Assay in Rats; OPPTS 870.5385 [84-2]; OECD 475

Report: IIA 5.8/11. Murli, H. (2009). Chromosomal aberrations in vivo in rat bone marrow cells with MON 52724. Covance Labs, Vienna, Va.; Monsanto report CV-09-078; September 1, 2009; unpublished; MRID 47899515

Dates of work: March 19,2009 - May 4, 2009

Guidelines: OECD 475(1997), EPA OPPTS 870.5385 (1998), MAFF (Shirasu, 1988) PMRA DACO 4.5.4

GLP: Yes OECD Principles of GLP, ENV/MC/CHEM (1998)
Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

GLP Exceptions: None

EXECUTIVE SUMMARY:

In an in vivo chromosome aberration test (MRID47899515), groups of five male CD (SD) IGS BR rats, were exposed once via oral gavage to MON 52724 (Purity 96.3%, Lot/Batch no. GLP-0903-19699-T), prepared in corn oil at doses of 0, 375, 750 or 1500 mg/kg. Bone marrow was harvested at 18 hours (all groups) and 42 hours (highest test group and the vehicle group) and examined for structural chromosomal aberration. Cyclophosphamide (CP) at 60 mg/kg was included as the positive control.

Following a toxicity range-finding study using 3 animals/sex/dose, the maximum dose tolerated (MTD) was estimated to be 1500 mg/kg, based on deaths at 1800 mg/kg (2 females) and 2000 mg/kg (1 M and 1 F). Only males were used in the main assay with oral gavage doses of 375, 750, and 1500 mg/kg because there were no clear differences in the toxic response between the sexes.

In the definitive study, only one male showed signs of irregular respiration at 1500 mg/kg. All other animals appeared normal. The MIs were not affected by treatment with the three test doses at the 24-hour harvest time. However, a slight but significant ($p \leq 0.05$) increase in the MI was recorded at the high dose, 42-hour harvest. Nevertheless, there was no significant increase in the percentage of cells with structural or numerical chromosome aberrations at any dose or harvest time. It is concluded that based on the data obtained from the study that MON 52724 did not cause increased numbers of chromosome aberrations in rat bone marrow cells at dose levels up to and including 1500 mg/kg by oral gavage. The expected response was induced by the positive control.

Based on these findings, it is concluded that MON 52724 did not cause increased numbers of chromosome aberrations in rat bone marrow cells at dose levels up to and including 1500 mg/kg in male rats in this study.

This study is classified as **totally reliable (acceptable/guideline)** and satisfies the guideline requirement for in vivo chromosme aberration data (OCSPP 870.5385; OECD 475).

MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** DCGA (MON 52724)
   Description: Off-White powder
   Lot/Batch#: GLP-0903-19699-T
   Purity: 96.3%
   CAS #: None given
   Stability of test compound: Listed with an expiration date of March 12, 2010.

2. **Control materials**
   Negative control: None
   Solvent control: Refined corn oil /10 mL/kg

   Positive control: Cyclophosphamide (CP, 60 mg/kg)
   CP was dissolved in sterile, deionized water

3. **Test system:**

Species: Rat
Strain: Sprague Dawley CD® (SD)IGS BR
Age: 8 weeks
Weight at dosing: 270 – 307g (initiation of treatment)
Source: Charles River Labs., Raleigh, NC
Acclimation period: 5 days
Diet: Harlan Teklan Certified Rodent Diet® # 2016C, *ad libitum*
Water: Tap water, *ad libitum*
Housing: Five males/sex/cage/ with bedding
No. of animals used per dose/time point: 3 Males 3 Females (Dose-range finding)
5 Males 0 Females (Main assay)

4. **Environmental conditions:**

Temperature: 18 – 26°C
Humidity: 30 – 70%
Air changes: 10 or more/hour
Photoperiod: 12 hours light / 12 hours darkness

5. Test compound concentrations used:

	Dose levels	Final volume	Route
Preliminary dose-range finding	500, 1000, 1500, 1800, 2000 mg/kg (3 M; 3F)	10 mL/kg	Oral gavage
Main study	0, 375, 750, 1500 mg/kg	10 mL/kg	Oral gavage

B. STUDY DESIGN AND METHODS:

1. Preliminary cytotoxicity assay: Doses for the main study were determined from the results of a toxicity study using only 3 mice per sex per dose and single oral gavage administrations of 500, 1000, 1800, 1500, and 2000 mg/kg of the test material. The animals were observed for 2 days and mortality and toxic signs were assessed.

2. Micronucleus assay:

Treatment and sampling times

a. Test compound and vehicle control

Dosing:	[X]	Once	[ ]	twice (24 hrs apart)			[ ]	Other			
Sampling (after last dose):	[ ]	6 hr	[ ]	12 hr	[X]	24 hr	[X]	42 hr High dose & vehicle	[ ]	72 hr	

b. Positive control

Dosing:	[X]	Once	[ ]	twice (24 hrs apart)			[ ]	Other			
Sampling (after last dose):	[ ]	6 hr	[ ]	12 hr	[X]	18 hr	[ ]	48 hr	[ ]	72 hr	

c. Details of slide preparation: At the appropiate harvest internal, groups of animals were sacrificed and bone marrow cells were collected, mixed with Hank's balanced salt solution and centrifuged. Cell pellets were resuspended in hypotonic KCl (0.075 M), fixed with a 3:1mixture of methanol and glacial acetic acid, and stained with 5% Giemsa.

a. Metaphase analysis

No. of cells examined per dose: If available, 100 per animalper sex for vehicle and treatment groups; 25/animal for the positive control were scored.

Scored for structural?	x	Yes		No
Scored for numerical?	x	Yes, polyploidy cells and endoreduplication		No
Coded prior to analysis?	x	Yes		No

Note: The mitotic index (MI) was determined from the count of cells in mitosis per 1000 cells.

b. Evaluation criteria:

Assay validity: The assay was considered valid if the structural aberration frequency was between 0 and the highest values in the historical control data (at 12-24 hours or 2.0 M at 42-48 hours) and the positive control group must produce a detectable increase over background.

Positive response: The test material was considered positive if it induced a significantly increased and dose-related for at least one dose.

d. Statistical analysis: The data were analyzed for significance using nonparametric ranked analysis techniques at $p \leq 0.05$.

II. RESULTS AND DISCUSSION

A. Analytical determinations: Analytical determinations were not conducted.

B. Preliminary dose-range finding test: One male and one female died at 2000 mg/kg and 2 of 3 females at 1800 mg/kg also died. Other signs of clinical toxicity observed at ≥1000 mg/kg included hypoactivity, irregular respiration, body tremors, and/or hunched posture (both sexes). Based on these findings, the high dose for the definitive study was 1500 mg/kg (males); females were not tested because there was no clear evidence of a sex-specific toxic response.

C. Cytogenetic Assay: In the main test, only one animal showed signs of toxicity at 1500 mg/kg (irregular respiration). All other animals appeared normal. Data from the evaluation of metaphases are summarized in Table 1 (Study Report Table 5). As shown, the MI was significantly ($p \leq 0.05$) increased at 1500 mg/kg, 42-hour harvest. The remaining doses for the 18-hour harvest did not affect the MIs. There, were, however, no significant increases in the percentage of cells with structural or numerical aberrations at any dose or harvest time. By contrast, the positive control (CP at 60 mg/kg) significantly ($p \leq 0.05$) altered the MI and the percentage of cells with structural chromosome aberrations.

Table 1. Chromosome Aberrations in Rat Bone Marrow - Summary

Study No. 8202711
Test Article: MON 52724 Initiation of Dosing: 04/07/2009

Treatment	Dose Level	Harvest Time (~ hr after last dose)	Number of Animals	Total Number of Cells Analyzed for Aberrations	% -g Group Mean ± S.E.	% +g Group Mean ± S.E.	Judgment (+/-)[a]	% Polyploidy Group Mean ± S.E.	% Endoreduplication Group Mean ± S.E.	Judgment (+/-)[b]	% Mitotic Index Group Mean ± S.E.
Controls											
Corn Oil	20 mL/kg	18	5	500	0.4 ± 0.40	1.4 ± 0.68		0.0 ± 0.00	0.0 ± 0.00		6.0 ± 1.30
		42	5	500	0.2 ± 0.20	0.6 ± 0.40		0.4 ± 0.40	0.0 ± 0.00		5.2 ± 0.84
Cyclophosphamide	60 mg/kg	18	5	250	71.2 ± 2.94	71.2 ± 2.94	+	0.0 ± 0.00	0.0 ± 0.00	-	1.1 ± 0.23[c]
Test Article											
	375 mg/kg	18	5	500	0.2 ± 0.20	0.4 ± 0.40	-	0.4 ± 0.24	0.0 ± 0.00	-	7.8 ± 0.87
	750 mg/kg	18	5	500	0.2 ± 0.20	0.2 ± 0.20	-	0.2 ± 0.20	0.0 ± 0.00	-	5.5 ± 1.24
	1500 mg/kg	18	5	500	0.2 ± 0.20	0.6 ± 0.24	-	0.2 ± 0.20	0.0 ± 0.00	-	6.1 ± 0.28
		42	5	500	0.0 ± 0.00	0.0 ± 0.00	-	0.4 ± 0.24	0.0 ± 0.00	-	8.4 ± 1.02[d]

% -g = % of cells with chromosome aberrations.
% +g = % of cells with chromosome aberrations + % of cells with gaps.
a Significantly greater in -g than the corresponding vehicle control, $p \leq 0.05$.
b Significantly greater in polyploidy than the corresponding vehicle control, $p \leq 0.05$.
c Mitotic index significantly less than the corresponding vehicle control, $p \leq 0.05$.
d Mitotic index significantly greater than the corresponding vehicle control, $p \leq 0.05$.
Source: Study Report Table 5, p 25 (MRID 47899515).

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U.S. EPA

CONCLUSIONS

MON 52724 was tested up to an adequate high dose, (1500 mg/kg), near or at the MTD but was not cytotoxic to the target organ and failed to induce a clastogenic response at any dose or sacrifice time. The positive control, CP at 60 mg/kg produced a significant decrease in the MI and a significant increase in structural chromosme aberrations ($p \leq 0.05$).

Accordingly, the study was negative and well-done; it is acceptable and satisfies OECD 475 and USEPA 870.5385 for an *in vivo* chromome aberration assay.

Deficiencies: None

Revised by U.S. Environmental Protection Agency

In Vivo Mammalian Cytogenetics – Erythrocyte Micronucleus Assay in Mice

Report: Xu, Y. (2007). In vivo mouse bone marrow micronucleus assay with MON 52708; Covance Laboratories Inc. Vienna, VA; Monsanto Study No. CV-2006-052; January 08, 2007; unpublished; MRID No. 47899511.

Dates of Work: May 4, 2006 to June 28, 2006

Guidelines: OPPTS 870. 5395 (August, 1998)
OECD 474 (July 21, 1997)
JMAFF –(Shirsu-1988)

GLP: Yes
GLP Exceptions: None

EXECUTIVE SUMMARY:

In an in vivo mouse bone marrow micronucleus assay (MRID 47899511), five male CD-1 (ICR) BR mice were exposed once by oral gavage to MON 52708 (Purity 97.9%; Lot/Batch No. GLP-0603-16958-T), prepared in corn oil at 0, 250, 500 or 1000 mg/kg. Bone marrow cells were harvested at 24 hours (all groups) and at 48 hours (vehicle and 1000 mg/kg) and examined for the ratio of polychromatic (PCEs) to normochromatic (NCEs) erythrocytes and the incidence of micronucleated PCEs (MPCEs)

The doses for the main study were based on the results of a dose-range finding studying in which groups of 3/sex/dose mice were administered 0, 500, 1000 or 2000 mg/kg and observed for up to 2 days for toxic signs and or mortality. Cyclophosphamide (80 mg/kg) was the positive control chemical. Based on mortality (1 M & 1F) at 2000 mg/kg, 1000 was selected as the maximum tolerated dose (MTD) and only males were used in the main assay since there was no apparent sex difference in toxicity.

All males in the main study appeared normal and remained healthy until sacrifice. The test chemical, at doses up to 1000 mg/kg, did not produce a cytotoxic response in the bone marrow at either the 24- or 48-hour harvest times. Similarly, there were no significant increases in the incidences of MPCEs for any treatment level at 24 or 48 hours.

Based on these considerations, it was concluded that under the conditions of this study, MON 52708 did not induce a clastogenic or aneugenic response in mouse bone marrow cells of male mice up to a level approaching the MTD.

This study is classified as **totally reliable (acceptable/guideline)** and satisfies the guideline requirement for In vivo Mouse Bone Marrow Micronucleus Assay of OCSPP 870.5395; OECD 474.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: MON 52708

Description: White powder
Lot/Batch#: GLP-0603-16958-T
Purity: 97.9%
CAS #: Not provided
Stability of test compound: Listed with an expiration date of March 20, 2007.

2. Control materials

Negative control: None
Vehiclet control: Corn oil; 10 mL/kg
Positive control: Cyclophosphamide: (CP) at 80 mg/kg

3. Test system:

Species: Male mouse; females were not used for the main study
Strain: CD-1® (ICR)BR
Age: 9 weeks
Weight at dosing: 30.0 – 38.7 g (at the initiation of treatment for th main assay)
Source: Charles River Labs. Portage, MI
Acclimation period: 5 days
Diet: PMI Certified Rodent Diet® # 5002, *ad libitum*
Water: Tap water, *ad libitum*
Housing: Five male mice/cage with bedding
No. of animals used per dose/time point: 3 Males 3 Females (Dose-range finding)
5 Males 0 Females (Main assay)

4. Environmental conditions:

Temperature: 64 – 79°F
Humidity: 30 – 70%
Air changes: 10 or more/hour
Photoperiod: 12 hours light / 12 hours darkness

5. Test compound concentrations used: Preliminary dose –finding toxicity assay:

	Dose levels	Final volume	Route
Preliminary dose-range finding	500, 1000 and 2000 mg/kg (3 M; 3F)	10 mL/kg	Oral gavage
Main study	0, 250, 500, 1000 mg/kg (5 M; 0F)	10 mL/kg	Oral gavage

B. STUDY DESIGN AND METHODS

1. In life dates: May 08, 2006 – May 26, 2006.

2. Preliminary cytotoxicity assay: Doses for the main study were determined from the results of a toxicity study using only 3 mice per sex per dose at 500, 1000, 2000 mg/kg of the test material in a single gavage dose. The animals were observed for 2 days and mortality and toxic signs were assessed.

3. Micronucleus assay:

Treatment and sampling times

a. Test compound and vehicle control

Dosing:	[X]	Once	[ ]	twice (24 hrs apart)			[ ]	Other		
Sampling (after last dose):	[ ]	6 hr	[ ]	12 hr	[X]	24 hr	[X]	48 hr (High dose & vehicle	[ ]	72 hr

b. Positive control

Dosing:	[X]	Once	[ ]	twice (24 hrs apart)			[ ]	Other		
Sampling (after last dose):	[ ]	6 hr	[ ]	12 hr	[X]	24 hr	[ ]	48 hr	[ ]	72 hr

4. Tissues and cells examined

Bone marrow	
No. of polychromatic erythrocytes (PCE) examined per animal:	2000
No. of total erythrocytes examined per animal:	500

5. Details of slide preparation – After 24 hours, 5 males were sacrificed in each dose level and at 48 hours a second set of 5 animals were sacrificed in the 1000 mg/kg and vehicle control groups. Bone marrow cells were extracted from the tibial bones of each animal, mixed with fetal bovine serum and centrifuged. Cell pellets were placed on slides and air-dried. Following methanol fixation, the slides were stained with a May-Grunwald and Giemsa solution and coded prior to counting. Prepared slides were examined for the incidence of micronucleated polychromatic erythrocytes (MPCE) and the ratio of PCE to total RBC.

***6.* Evaluation criteria** – The study was considered valid if the frequency of MPCEs and the percentage of PCEs in both the negative control group was within the historical control acceptable range. In addition, the frequency of MPCEs in the positive control group should be markedly and significantly increased relative to the negative control group. Historical control data were provided by the performing laboratory.

7. Statistical methods – As stated in the Study Report, "The following statistical methods were used to analyze the micronucleus data:.

- "Assay data analysis was performed using an analysis of variance (Winer, 1971) on untransformed proportions of cells with micronuclei per animal and on untransformed, PCE:NCE ratios when the variances were homogeneous. Ranked proportions were used for heterogeneous variances."
- "If the analysis of variance was statistically significant ($p \leq 0.05$), Dunnett's t-test (Dunnett, 1955; 1964) was used to determine which dose groups, if any, were statistically significantly different from the vehicle control. Analyses were performed separately for each sampling time."

II. RESULTS AND DISCUSSION

A. Analytical determinations: It was reported that actual concentrations were not determined in the study.

B. Preliminary (Dose range-finding) test: The results of the range-finding study are summarized in the Table 1. As shown, 2000 mg/kg was lethal for 1 male and 1 female mouse on day 1. Additionally, one female mouse at 1000 mg/kg and 1 male mouse on day 2 at 500 mg/kg were found dead. Clinical signs of hypoactivity, squinted eyes and hunched posture were noted in the female at the lower dose and hypoactivity and limited use of hind limbs was recorded for a single female at the 2000 mg/kg. The authors concluded that 1000 mg/kg was the maximum tolerated dose (MTD) and that there was no relevant difference in toxicity between the sexes.

Table 1 – Clinical Observations Dose Range-finding Study

Target Dose Level (mg/kg)	Sex	Animal ID	Time After Dosing				
			IPD	~0.5 hour PD	~1 hour post dose	1 day	2 days
500	Male	2157	0	NP	0	0	0
		2159	0	NP	0	0	6
		2166	0	NP	0	0	0
	Female	2174	0	NP	0	0	0
		2177	0	NP	0	0	0
		2179	0	NP	0	0	0
1000	Male	2158	0	NP	0	0	0
		2163	0	NP	0	0	0
		2167	0	NP	0	0	0
	Female	2169	0	NP	0	0	0
		2171	0	NP	0	0	0
		2176	0	NP	0	1, 4, 5	6
2000	Male	2162	0	1, 2	3	0	0
		2164	0	0	0	0	0
		2165	0	0	0	6	-
	Female	2168	0	0	0	0	0
		2170	0	0	0	0	0
		2172	0	1 ,2	0	6	-

Key: 0 = Normal, 1 = hypoactive, 2 = limited use of hind limbs, 3 = slightly hypoactive, 4 = squinted eyes, 5 = hunched posture, 6 = found dead
IPD = Immediately post dosing
PD = Post dosing
NP = Not performed
Source: Study Report Table 1, p.20 (MRID 47899511)

C. Micronucleus assay: The male mice in all test dose groups appeared normal after dosing and remained as such for the remainder of the assay. Examination of the slides for the occurrence of micronuclei following the oral gavage doses of 250, 500, 1000 or cyclophosphamide at 80 mg/kg and exposure for either 24 or 48 hours is summarized in Table 2. As shown, no significant differences in the PCE:NCE ratios or the frequency of MPCEs was seen in any treatment group at 24 hours or in the high-dose group at 48 hours. Only CP induced a significant ($p \leq 0.05$) increase of 2.09% in the MPCEs.

Table 2 - Micronucleus Assay Summary

Treatment	Dose	Harvest Time	% Micronucleated PCEs Mean of 2000 per Animal ± S.E. Males	Ratio PCE:NCE Mean ± S.E. Males
Controls				
Vehicle	Corn Oil 10 mL/kg	24 hr	0.03 ± 0.01	0.89 ± 0.04
		48 hr	0.04 ± 0.01	0.83 ± 0.06
Positive	CP 80 mg/kg	24 hr	2.09 ± 0.21*	0.50 ± 0.05**
Test Article	250 mg/kg	24 hr	0.04 ± 0.02	0.85 ± 0.03
	500 mg/kg	24 hr	0.05 ± 0.02	0.78 ± 0.05
	1000 mg/kg	24 hr	0.04 ± 0.02	0.91 ± 0.03
		48 hr	0.03 ± 0.01	0.81 ± 0.06

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
** Significantly less than the corresponding vehicle control, $p \leq 0.05$.
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

Source: Study Report Table 3, p.21 (MRID 47899511)

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:

RELIABILITY RATING: Totally reliable
This study is generally compliant with OECD 474 (1997)

C. CONCLUSIONS: MON 52708 was tested up to an adequately high dose, (1000 mg/kg) that approached the MTD but did not affect the PCE:NCE ratio or produce a significant increase in MPCEs at any dose or sacrifice time. The positive control, CP at 80 mg/kg induced a significant increase in MPCEs, thus demonstraitng the sensitivity to the test system to detect micronucleus induction.

Based on these considerations, it was, therefore, concluded that MON 52708 was negative in this mouse bone marrow micronucleus test system in a well-conducted study.

Deficiencies: None

Revised by U.S. Environmental Protection Agency

***In Vivo* Mammalian Cytogenetics – Erythrocyte Micronucleus Assay in Mice; OCSPP 870.5395 ['84-2]; OECD 474.**

Report: IIA 5.4.2/01. In vivo mouse bone marrow micronucleus assay with MON 11900, Xu, Y. (2007). Covance Laboratories Inc. Vienna, Virginia; Monsanto Study No. CV-2006-084, April 19, 2007, unpublished; MRID No. 47899528.

Dates of work: September 05, 2006 - September 21, 2006

Guidelines: OPPTS 870. 5395 (August, 1998)
OECD 474 (July 21, 1997)
JMAFF –(Shirsu-1988)

GLP: Yes USEPA Principles of GLP, ENV/MC/CHEM (1989)
Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

GLP: Exceptions None

Revised by U.S. Environmental Protection Agency

EXECUTIVE SUMMARY:

In an in vivo mouse bone marrow micronucleus assay (MRID 47899528), groups of CD-1 male mice were exposed once by oral gavage to MON 11900 (Purity 94.6%; Lot/Batch No. GLP-0604-17184-T), prepared in corn oil at doses of 0, 250, 500 and 1000 mg/kg. Bone marrow cells were harvested at 24 hours (all groups) and at 48 hours (vehicle and 1000 mg/kg) and examined for the ratio of polychromatic (PCEs) to normochromatic (NCEs) erythrocytes and the incidence of micronucleated PCEs (MPCEs)

The doses for the main study were based on the results of a dose-range finding studying in which one male, receiving 1000 mg/kg, died within the first hour of dosing. No other deaths or were observed at any dose (500, 1000 and 2000 mg/kg). At the high dose, toxic signs included: hypoactivity, irregular respiration, convulsions (males only), squinted eyes, and/or ataxia in both sexes. Accordingly, 1000 mg/kg was selected as the maximum tolerated dose (MTD) and only males were tested because a sex-related difference in the toxic response was not seen.

In the micronucleus assay, no deaths occurred at the high dose but signs of hypoactivity, squinted eyes, ataxia, and convulsions were noted during the first hour post-treatment. All animals appeared normal by day 2. There were no significant increases in the incidences of MPCEs for treatment levels of 250, 500 mg/kg at 24 hours. An exception being the 24- hour harvest of the 1000- mg/kg group, which produced a significant increase ($p \leq 0.01$) in the percentage of MPCEs (0.08), compared to 0.02 MPCEs for the vehicle. However, the 48- hour examination of the high dose group yielded a negative response. Additionally, the micronuclei rate for the 1000- mg/kg group at 24 hours was within the historical control range (0.00-0.25 % MPCEs) of the performing laboratory.

Based on these considerations, it was concluded that, MON 11900 was neither clastogenic nor aneugenic in the mouse bone marrow.

This study is classified as **totally reliable (acceptable/guideline)** and satisfies the guideline requirement for in vivo cytogenetic mutagenicity data (OCSPP 870.5395; OECD 474).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: MON 11900

Description: White powder
Lot/Batch#: GLP-0604-7184-T
Purity: 94.6%
CAS #:
Stability of test compound: Stability information not presented but an expiration date of April 26, 2007 was provided.

2. Control materials

Negative control: None
Vehicle control: Corn oil; 10 mL/kg
Positive control: Cyclophosphamide (CP) at 80 mg/kg

3. Test system:

Species: Male mouse; females were not used for the main study
Strain: CD-1® (ICR)BR
Age: 8 weeks
Weight at dosing: 34.2 – 39.8 g (at the initiation of treatment)
Source: Harlan, Frederick, MD
Acclimation period: 5 days
Diet: PMI Certified Rodent Diet® # 5002, *ad libitum*
Water: Tap water, *ad libitum*
Housing: Five male mice/cage with bedding
No. of animals used per dose/time point: 3 Males 3 Females (Dose-range finding)
5 Males 0 Females (Main assay)

4. Environmental conditions:

Temperature: 64 – 79°F
Humidity: 30 – 70%
Air changes: 10 or more/hour
Photoperiod: 12 hours light / 12 hours darkness

5. Test compound concentrations used:

	Dose levels	Final volume	Route
Preliminary dose-range finding	500, 1000 and 2000 mg/kg (3 M; 3F)	10 mL/kg	Oral gavage
Main study	0, 250, 500, 1000 mg/kg (5 M; 0F)	10 mL/kg	Oral gavage

B. STUDY DESIGN AND METHODS

1. In life dates: September 05, 2006- September 21, 2006

2. Preliminary cytotoxicity assay: Doses for the main study were determined from the results of a toxicity study using only 3 mice per sex per dose at 500, 1000, 2000 mg/kg of the test material in a single gavage dose. The animals were observed for 2 days and mortality and toxic signs were assessed.

3. Micronucleus assay:

Treatment and sampling times

a. Test compound and vehicle control

Dosing:	[X] Once	[ ] twice (24 hrs apart)	[ ] Other
Sampling (after last dose):	[ ] 6 hr	[ ] 12 hr	[X] 24 hr
	[X] 48 hr (High dose & vehicle)	[ ] 72 hr	

b. Positive control

Dosing:	[X] Once	[ ] twice (24 hrs apart)	[ ] Other
Sampling (after last dose):	[ ] 6 hr	[ ] 12 hr	[X] 24 hr
	[ ] 48 hr	[ ] 72 hr	

4. Tissues and cells examined

Bone marrow	
No. of polychromatic erythrocytes (PCE) examined per animal:	2000
No. of total erythrocytes examined per animal:	500

5. Details of slide preparation – After 24 hours, 5 males were sacrificed in each dose level and at 48 hours a second set of 5 animals were sacrificed in the 1000 mg/kg and vehicle control groups. Bone marrow cells were extracted from the tibial bones of each animal, mixed with fetal bovine serum and centrifuged. Cell pellets were placed on slides and air-dried. Following methanol fixation, the slides were stained with a May-Grunwald and Giemsa solution and coded

prior to counting to control for bias. Prepared slides were examined for the incidence of micronucleated polychromatic erythrocytes (MPCE) and the ratio of PCE to total RBC.

6. **Evaluation criteria** – The study was considered valid if the frequency of MNPCE and the percentage of PCE in both the negative and positive control groups were within the historical control range, provided by the sponsor. In addition, the frequency of MPCE in the positive control group should be markedly and significantly increased relative to the negative control group.

7. Statistical methods – As stated in the Study Report, "The following statistical methods were used to analyze the micronucleus data:.

- "Assay data analysis was performed using an analysis of variance (Winer, 1971) on untransformed proportions of cells with micronuclei per animal and on untransformed, PCE:NCE ratios when the variances were homogeneous. Ranked proportions were used for heterogeneous variances."
- "If the analysis of variance was statistically significant ($p \leq 0.05$), Dunnett's t-test (Dunnett, 1955; 1964) was used to determine which dose groups, if any, were statistically significantly different from the vehicle control. Analyses were performed separately for each sampling time."

II. RESULTS AND DISCUSSION

A. Analytical determinations: It was reported that actual concentrations were not determined in the study.

B. Preliminary (Dose range-finding) test: The results of the range-finding study are summarized in Table 1 (Study Report Table 1). As shown, no deaths were observed in either sex at 2000 mg/kg. However, 1000 mg/kg was lethal for 1 male at 1 hour post dosing; no further unscheduled deaths were recorded in the study. Clinical signs of hypoactivity, irregular respiration, convulsions, squinted eyes and hunched posture were noted in several animals in both sexes at 1000 mg/kg and 2000 mg/kg. The authors concluded that 1000 mg/kg was the maximum tolerated dose (MTD) and that there was no relevant difference in toxicity between the sexes.

IIIA 10.1.7 Table 1 Clinical Observation – Dose Range-finding Study

Target Dose Level (mg MON 11900/kg body weight)	Sex	Animal ID	Time After Dosing				
			IPD	1 hour PD	3 hours PD	1 day	2 days
500	M	4396	0	1	NP	0	0
		4394	0	1	NP	0	0
		4399	0	1	NP	0	0
	F	4403	0	0	NP	0	0
		4404	0	0	NP	0	0
		4405	0	0	NP	0	0
1000	M	4393	0	1	8	0	0
		4400	0	2	-	-	-
		4401	0	3,4,5,6	8	0	0
	F	4406	0	3,4,5,6,7	8	0	0
		4411	0	1	8	0	0
		4414	0	1	8	0	0
2000	M	4397	0	3,4,6,7	1,6	0	0
		4398	0	3,4,6,7	6,8	0	0
		4402	0	3,4	6,8	0	0
	F	4407	0	3,4,5,6,7	1,6,8	0	0
		4410	0	3,4,5,7	8	0	0
		4412	0	3,4,5,6,7	6,8	0	0

Key: 0 = Normal, 1 = slightly hypoactive, 2 = found dead, 3 = hypoactive, 4 = irregular respiration, 5 = convulsions, 6 = squinted eyes, 7 = ataxia, 8 = hunched posture
IPD = Immediately post dosing PD = Post dosing
NP = Not performed
Source: Study Report Table 2, p 23 (MRID 47899528)

C. Micronucleus assay: Immediately after dosing most animals in the lower doses exhibited slight hypoactivity, but were generally normal at 1 hour post dosing. The highest dose tested (1000 mg/kg) showed toxic effects immediately after dosing and up to 1 hour. These effects included: hypoactivity, squinted eyes, ataxia, and convulsions were noted during the first hour post-treatment. All animals appeared normal by day 2.

Data from all treatment groups at the 24- and 48-hour harvest are summarized in Table 2 (Study Report Table 5). As shown, the PCE:NCE ratio was not affected at any dose or harvest time. However, 24 hours after exposure to 1000 mg/kg, a significant ($p \leq 0.01$) increase in the percentage of micronuclei was reported (0.08 % MPCEs). This value was approximately 4X greater than the vehicle control value for the same time period (0.02 % MPCEs). Nevertheless, the increase was not seen at 48 hours and was within the historical control range (0.00-0.25 % MPCEs for the 24-hour period) of the performing laboratory. No appreciable increases were seen for the other doses. It was, therefore, concluded that the increase was spurious.

By contrast, the positive control (CP, 80 mg/kg) induced a significant ($p \leq 0.01$) increase of 1.59% in the percentage of MPCEs.

Table 2: Micronucleus Assay – Summary Table

Assay No.: 28354-0-455OECD
Test Article: MON 11900
Initiation of Dosing: 19 September 2006

Treatment	Dose	Harvest Time	% Micronucleated PCEs Mean of 2000 per Animal ± S.E. Males	Ratio PCE:NCE Mean ± S.E. Males
Controls				
Vehicle	Corn Oil 10 mL/kg	24 hr	0.02 ± 0.01	0.59 ± 0.04
		48 hr	0.04 ± 0.02	0.66 ± 0.10
Positive	CP 80 mg/kg	24 hr	1.59 ± 0.29*	0.57 ± 0.04
Test Article	250 mg/kg	24 hr	0.02 ± 0.01	0.57 ± 0.04
	300 mg/kg	24 hr	0.03 ± 0.01	0.56 ± 0.07
	1000 mg/kg	24 hr	0.08 ± 0.02**	0.49 ± 0.03
		48 hr	0.03 ± 0.02	0.46 ± 0.03

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
** Significantly greater than the corresponding vehicle control, $p \leq 0.05$.
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte
The vehicle control was corn oil and the negative control was cyclophosphamide.
Source: Study Report, Table 5, p. 26 (MRID 47899528)

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:
RELIABILITY RATING: Totally reliable

This study is compliant with OECD 474 (1997)

C. CONCLUSIONS: MON 11900 was tested up to an adequate MTD (1000 mg/kg), which did not affect the PCE:NCE ratio but did produce a significant ($p \leq 0.01$) increase in the percentage of micronuclei (0.08 % MPCEs) at 1000 mg/kg at 24 hours only. This value was approximately 4X greater than the vehicle control value for the same time period (0.02 % MPCEs). However, the increase was not seen at 48 hours and was within the historical control range (0.00-0.25 % MPCEs for the 24-hour period) of the performing laboratory. No appreciable increases were seen for the other doses. We agree, therefore, with the study author's conclusion that the increase was spurious. The positive control, cyclophosphamide at 80 mg/kg produced a significant ($p \leq 0.05$) increase in the frequency of MPECs, thus demonstration the sensitivity of the assay to detect micronucleus induction.

Based on these considerations, it was concluded that MON 11900 was negative up to 1000 mg/kg in this well-done mouse bone marrow micronucleus assay.

IIIA 10.1.8 Deficiencies: None

Revised by U.S. Environmental Protection Agency

Study Type: 90- day Oral Toxicity / Neurotoxicity

Report: IIA 5.8/7. Kirkpatrick, J. B. (2011). Amended Report: A 90-Day Oral (Diet) Study of MON 11900 in Rats. WIL Research Laboratories, LLC. Ashland Ohio. Sponsor: Monsanto Co., St. Louis, MO, Sponsor Study No. WI-2006-015, MRID No. 48358001, unpublished.

Dates of work: June 13, 2006- October 25, 2006

Guidelines: OECD 408 and section 424, EPA OPPTS 870.3100/870.6200
Deviations: none significant.
PMRA DACO 4.3.1

GLP: Yes

Executive Summary:

In a 90-day oral toxicity/neurotoxicity study in Sprague-Dawley (Crl:CD® [SD] rats (MRID 48358001), groups of 16 rats/sex were dosed with Dicamaba (MON 11900) in daily diets with either 0, 500, 3000, 6000, or 12000 ppm test material, which corresponded to 0, 34, 197, 397, 803 mg/kg/day in males and 0, 39, 230, 458, 938 mg/kg/day in females. There were 6 animals /sex dose in subset A and B and 4/sex/dose in subset C. Subsets A and B were used for the functional observational battery (FOB) and subsets B and C were used for clinical and pathology determinations.

There were small body weight changes only in males. At the end of the study, 12000 ppm males weighed 5% less than controls with a cumulative weight gain of 9% less than controls; neither value was statistically significant.

Other than one death in a control male; all animals survived to sacrifice. Clinical observations in 12000 ppm males included unkempt appearance (2/16 males, vs 0/16 controls) and gasping/rales (1/16 males, 4 occurrences, vs 0/16 controls). Uncoordinated righting ability was noted in 3/12 males in the 12000 ppm group. There was also lower hindlimb footsplay in 12000 ppm males during week 7. Females in the 12000 ppm group had rigid muscle tone (6/16 females) and one of these showed an impaired equilibrium on 2 different times. Motor activity was unaffected by treatment.

The NOAEL for MON 11900 is 397 mg/kg/day and the LOAEL is 803 mg/kg/day based on FOB and clinical observations (rigid muscle tone, impaired equilibrium, uncoordinated righting ability, and decreased lower hindlimb footsplay). This study is classified totally reliable (acceptable/guideline) and satisfies the guideline requirements for a 90-day rat toxicity study and neurotoxicity study (OECD 408 and EPA OPPTS 870.3100/870.6200).

MATERIALS AND METHODS

A. MATERIALS

1. Test material: MON 11900

Description:	Irregular off-white flakes
Lot/Batch#:	GLP-0604-17184-T
Purity:	94.6%
CAS #:	None given
	dos
Stability of test compound:	Samples had 85% to 115% of target conc., were homogeneous, and stable for 4 days at room temp. and for 31 days frozen

6. Test compound concentrations used

Test material was added to acetone and mixed with the rodent diet (PMI rodent # 5002 meal)
to produce dose levels of 0, 500, 3000, 6000, or 12000 ppm.

7. Environmental Conditions:
Mean daily temperature: 67.7 °F to 71.8°F
Relative Humidity: 41.8% to 72.3%
There was 12 hour light/dark photoperiod and at least 10 fresh air changes per hour.
Water and diet were supplied *ad libitum*. Water was from reverse osmosis treated tap water.

B. STUDY DESIGN AND METHODS:

There were 16 animals/sex/test level (Groups 1-5); treatment was for 13 consecutive weeks. Each dose group was subdivided into sets A (6 rats/sex), B (6 rats/sex), C (4 rats/sex).

Set A rats (6 rats/sex) were perfused for neuropathology evaluations. Sets A and B were used for FOB and motor activity (total of 12 rats/sex). Set B and Set C (total 10 rats) were evaluated for clinical pathology, organ weights and anatomic pathology. The rats were approximately 7 weeks old at start of dosing. Body weights ranged from 205 to 268 grams and 153 to 196 grams in males and females respectively.

Functional observational battery (FOB) testing was performed pretest and again at weeks 3, 7, and 12, using the protocols of Moser et al., 1991, Irwin, 1968, Gad, 1982 and others and included home cage observations, handling observations, open field observations, sensory observations, neuromuscular observations, and physiological observations. Animals were observed for posture, convulsions, feces consistency, biting, eyelid closure, eye prominence, ease of removal from cage, ease of handling in hand, lacrimation/ chromodacryorrhea, piloerection, red/crusty deposits, salivation, fur appearance, respiratory rate and character, mucous membranes/eyes/skin color, muscle tone, mobility, rearing,

convulsions, grooming, bizarre/stereotypic behavior, time to first step, gait, arousal, urination/defecation, gait score, backing, approach response, startle response, pupil response, forelimb extension, righting reflex, touch response, tail pinch response, eyeblink response, hindlimb extension, olfactory orientation, extensor strength, foot splay, grip strength, Rotarod performance, catalepsy, body temperature, and body weight.

Clinical Pathology: Sets B and C were used to collect blood and urine samples both prior to and at 13 weeks of study. Blood was obtained from the retro-orbital sinus following isoflurane sedation. Hematology parameters included WBC, RBC, platelets, clotting times, morphology, MCV, MCH, MCHC, reticulocyte count, and differentials.

Chemistry analyses included total bilirubin, urea nitrogen, creatinine, alkaline phosphatase, ALT, AST, albumin, total protein, globulin, A/G ratio, cholesterol, calcium, chloride, phosphorus, potassium, sodium.

C. Positive Control Results for Neurotoxicity:

Positive control results for neurotoxicology measurements are reported in Appendix F of the WIL-50306 report (page 1003). This appendix contains summaries of four validation studies conducted by WIL (WIL 99140, WIL 99149, WIL 99263, and WIL 99310). Briefly, these were as follows:

The purpose of the WIL-99140 study was to determine the appropriate length of time for their locomotor activity measurements based on the requirement that rats should approach normal activity levels by the last 20% of the session. The increase in activity measured in this study was due to the increased activity level that occurs in animals in a novel environment.

The purpose of the WIL -99149 study was to demonstrate the sensitivity of the SDI-PAS system for detecting alterations in locomotor activity in rats. Two compounds known to alter motor activity in rats: d-Amphetamine sulfate treatment which elicit increases in motor activity, and chlorpromazine hydrochloride which decreases motor activity. Results of this study indicated that the SDI-PAS system was sufficiently sensitive to detect dose-related increases and decreases in locomotor activity.

The purpose of WIL-99263 was to train personnel and assess inter-observer reliability in performing Functional Observational Battery Assessments (FOB). This study involved the use of two positive control compounds (3.3'-Iminodipropionitril (IDPN) and Parathion). Data are presented showing the sensitivity of the assessment of effects of these compounds on neurological effects of the test substances and the reliability between observers.

The purpose of WIL-99310 was to train personnel and assess inter-observer reliability of reported FOB results for neurotoxicity studies. Three positive control compounds were used (3.3'-Iminodipropionitril (IDPN), Parathion and d-Amphetamine). Corn oil was

used as a negative control. The performance of the observers was consistent and deemed acceptable for detecting neurotoxicity effects.

II. RESULTS AND DISCUSSION

Observations: There was one death at day 16 in a control male. All other animals survived to sacrifice. Clinical observations in 12000 ppm males included unkempt appearance (2/16 males, vs 0/16 controls) and gasping/rales (1/16 males, 4 occurrences, vs 0/16 controls). Females in the 12000 ppm group had rigid muscle tone (6/16 females), and one of these showed an impaired equilibrium on 2 different times. These effects did not correlate with effects noted in the functional observation battery.

FOB and Motor Activity: Uncoordinated righting ability was noted in 3/12 males in the 12000 ppm group. There was also lower hindlimb footsplay in 12000 ppm males during week 7. These effects were not noted during week 3 or week 12. Other FOB observations were similar among the different groups or did not exhibit a dose response. Motor activity was unaffected by treatment.

Body Weight: The test material produced small body weight changes only in males (see Table 4). At week 13, high-dose males weighed 5% less than controls and cumulative weight gain was 9% less than controls; neither value was statistically significant.

Table 1 – Summary of Body Weights and Weight Gain (g) for Males						
Group		0 PPM	500 PPM	3000 PPM	6000 PPM	12000 PPM
Body Weights						
Week 0	Mean	235	237	232	232	233
	S.D.	14.3	12.9	13.3	12.0	14.2
Week 12	Mean	548	560	530	529	522
	% difference from control	--	2.2%	-3.3%	-3.5%	-4.7%
	S.D.	61.6	47.1	51.9	43.1	55.8
Week 13	Mean	561	575	541	538	531
	% difference from control	--	2.5%	-3.6%	-4.1%	-5.3%
	S.D.	60.5	48.8	52.4	44.4	57.6
Cumulative Weight Gain						
Weeks 0-13	Mean	327	338	309	306	298
	% difference from control	--	3.4	-5.5	-6.4	-8.9
	S.D.	50.8	44.1	43.3	35.2	48.0

Source: Pages 101, 104, 119 of study report

Food consumption: Except for the first week of the study for high-dose males, food consumption was generally comparable in the different dose groups.

Compound consumption:

Table 2 – Summary of Calculated Test Substance Consumption			
Group	Target Dietary Level (ppm)	Mean Calculated Test Substance Consumption (mg/kg/day)[a]	
		Males	Females
1	0	0	0
2	500	34	39
3	3000	197	230
4	6000	397	458
5	12000	803	938

a = Represents the grand mean calculated over 90 days.
Source: Page 47 of Report

Clinical Pathology:

Hematology: Total white cell counts and absolute lymphocyte counts were slightly increased in females receiving 12000 ppm of test material. These values were within the historical control range for the testing laboratory. All other hematological parameters were not significantly different from controls in either sex treated.

Table 3 – Summary of Hematology Values in Females

Analysis	**Group**	**0 PPM**	**500 PPM**	**3000 PPM**	**6000 PPM**	**12000 PPM**
White Cells (thous/µL)	Mean	5.30	4.57	6.50	6.38	8.04*
	% difference from control	--	-13.8%	22.6%	20.4%	51.7%
	S.D.	1.579	0.969	3.161	1.952	2.346
Red Cells (mil/µL)	Mean	8.33	8.31	8.38	8.37	8.35
	% difference from control	--	-0.2%	0.6^	0.5%	0.2%
	S.D.	0.181	0.136	0.175	0.284	0.276
Hemoglobin (g/dL)	Mean	15.5	15.2	15.3	15.1	15.3
	% difference from control	--	-1.9%	-1.3%	-2.6%	-1.3%
	S.D.	0.49	0.13	0.29	0.64	0.41
Hematocrit (%)	Mean	44.7	44.4	44.6	44.4	44.6
	% difference from control	--	-0.7%	-0.2%	-0.7%	-0.2%
	S.D.	1.31	1.01	0.89	1.51	1.60

*= Significantly different from the control group at 0.05 using Dunnett's test
Source: Page 249 of Report

Serum Chemistries: The males at 12000 ppm had a statistically significant increased A/G ratio (p=0.05), due to decreased globulins. There were slight elevations in alkaline phosphatase in 12000 males (+46%) and females (+30%). Both of these effects are of minor toxicological significance. Other variations in clinical chemistries (elevated phosphorus in females at 6000, and 12000 ppm, higher chloride at 500 ppm, lower protein levels in females at 500 and 6000 ppm., lower cholesterol at 500, 3000, and 6000 ppm in females) either did not show a dose-response relationship or are of uncertain toxicological significance.

Urinalysis: There were no statistical differences in the urine values found when comparing treated to controls in the study.

Ophthalmic Examinations: All findings were normal and not indicative of treatment effects.

Pathology: Brain length was decreased in 12000 ppm males by 3.6% in comparison to controls, which was within the historical control range. This is not considered a treatment-related effect because treatment began at age 55 days, by which time major development of the brain was already complete.

Table 4 – Summary of Brain Weights (g) and Measurements (mm) in Males

Analysis	**GROUP:**	**0 PPM**	**500 PPM**	**3000 PPM**	**6000 PPM**	**12000 PPM**
Brain Weight	Mean	2.17	2.18	2.14	2.14	2.08 (-4.1%)
	S.D.	0.034	0.075	0.136	0.052	0.074
Brain Length	Mean	22.3	22.0	22.4	21.9	21.5* (-3.6%)
	S.D.	0.33	0.43	0.40	0.29	0.68
Brain Width	Mean	15.5	15.6	15.6	15.7	15.4
	S.D.	0.31	0.26	0.37	0.08	0.18

*= Significantly different from the control group at 0.05 using Dunnett's test
Source: Page 285 of Report

There were no histopathological test material related changes. The incidence, severity, or histological character of any findings was considered spontaneous, and incidental.

Organ Weights: The absolute and relative liver weights of the 12000 ppm group females were increased. Each was elevated by 10.4% and 10.2% respectively. There was no histopathologic evidence in the liver tissues which would support the cause of the slightly elevated serum alkaline phosphatase levels in the females. However the males at 12000 ppm did not exhibit increased liver weights even though the serum enzyme level was higher than in females. Therefore, the increased liver weight in females is not considered toxicologically significant. The testes of the males at 6000 ppm were heavier than controls but were considered an isolated, spurious event.

II. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U.S. EPA

This study report, MRID 48358001, updates MRID 47899529 to correct units for brain measurement in Tables 45 and 96 in order to report absolute, rather than adjusted measurements.

The neurological effects were sometimes only observed sporadically, but were attributed to treatment because they were observed in the high-dose group and were consistent with effects occuring at similar doses in a 1994 subchronic neurotoxicity study with dicamba (MRID 43245210, D204480).

Clinical pathology changes were of generally minor toxicological significance.

Reliability Rating: This study is classified totally reliable (acceptable/guideline) and satisfies the guideline requirements for a 90-day rat toxicity study and neurotoxicity study (OECD 408 and EPA OPPTS 870.3100/870.6200).

Deficiencies: There were no deficiencies for this study.

Revised by U.S. Environmental Protection Agency

Study Type: Metabolism and Pharmacokinetics

Report: IIA 5.8/1. Shah, J.F., McClanahan, R.H. (2009b). Metabolism of [^{14}C]DCSA in Sprague-Dawley Rats. Ricerca Biosciences, LLC, unpublished report No. MSL-20328/019913-1-1, study No. 06-98-M-3/XX-09-305. MRID 47899502. Unpublished.

Dates of Work: July 12, 2006 – September 30, 2009

Guidelines: OPPTS 870.7485 Tier 1
Deviations: None
OECD 417 (partial, single oral dose in males only)
Deviations: None
PMRA DACO 4.5.9

GLP: Yes

Executive Summary: In a metabolism and pharmacokinetic study (MRID 47899502), a mixture of radiolabelled and unlabelled DCSA (5,560 dpm/µg) as a suspension in corn oil was administered by oral gavage individually to six male rats (Sprague-Dawley Crl:CD® (SD)) as a single dose at a target dose level of 100 mg/kg bw.

[^{14}C]DCSA was extensively absorbed by the rat and rapidly excreted with very little retention in tissues. Urinary excretion was the major route of elimination accounting for approximately 95% of the administered dose. Levels of radioactivity in the tissues after 7 days were very low with kidney containing the highest levels of the dose. Limited metabolism of DCSA occurred in the rat *via* glucuronidation at either the carboxylic acid or phenol moiety. Unchanged DCSA represented approximately 82% of the dose.

This study is classified totally reliable (acceptable/guideline) and satisfies the guideline requirement for a metabolism/pharmacokinetic study when evaluated with MRID 47899503.

Test conditions:

[Phenyl-U-^{14}C]DCSA (3,6-dichloro-2-hydroxybenzoic acid, structure in Figure 5.8-1) (Lot No. 6116-01A, radiochemical purity 98.8%, chemical purity 99.5%, specific activity 35.2 mCi/mmol, 6.29 MBq/mg, 377,507 dpm/µg); unlabelled DCSA (Lot No. GLP-0603-16959-T, purity 98.2%).

Figure 5.8-1: Structure of [^{14}C]DCSA test material

* Denotes uniform ^{14}C ring labelling

[EMBED ChemDraw.Document.5.0]

A mixture of radiolabelled and unlabelled DCSA (5,560 dpm/µg) as a suspension in corn oil was administered by oral gavage individually to six male rats (Sprague-Dawley Crl:CD® (SD)) as a single dose at a target dose level of 100 mg/kg bw. Approximately 2 mL/kg bw of dose suspension and approximately 250 µCi/kg bw of radioactivity were administered to each animal. Rats were 57-59 days old and weighed 229-266 g at the time of dosing. Two animals (Group 1) were subjects in an Expired Air Metabolism experiment while the remaining four animals (Group 2) were subjects in an Excretion/Mass Balance experiment as outlined in Table 5.8-1.

Table 5.8-1: Study design

Test Group	Dose (mg/kg)	Animals (No./Sex)	Dosing Route	Sample Collection
1	100	2 Male	Oral	Excreta, expired air
2	100	4 Male	Oral	Excreta, tissues, blood

Expired volatiles were collected at 6 h, 12 h, 24 h and 48 h post-dose (Group 1 only). Trapping of expired air was discontinued after 48 h since less than 1% of the administered dose was found in the expired air traps by 48 h. Excreta (urine, faeces and cage washes) were collected from all animals at 6 h, 12 h and 24 h after dosing followed by 24 h interval collections thereafter until termination by exsanguination at 7 days. Collection and analysis of excreta from animals of Group 1 was conducted only to verify the dose received and to confirm an acceptable mass balance for the Expired Air experiment. Tissues (liver, fat, GI tract with contents, kidney and spleen), whole blood and residual carcass were collected at termination (Group 2 only). Urine, faeces, cage washes, tissues, blood and carcass were analyzed for radioactivity content. Metabolite profiles were generated for pooled samples of urine, faeces and cage washes of Group 2. This study fulfils the OPPTS 870.7485 Tier 1 guideline. Although this study only partially fulfils the OECD 417 guideline, together with the pharmacokinetic and metabolism study (study 5.8.1/2) utilizing repeat dosing of both male and female rats, a rather complete picture of the toxicokinetics of DCSA was obtained.

Analytical methods:

Radioactivity in all samples was quantified by liquid scintillation counting (LSC). Urine and cage wash samples were analyzed directly by LSC. Faeces, liver, kidney and GI tract samples were homogenized with water and aliquots were analyzed by LSC after solubilization. Spleen and fat samples, and residual carcasses, were solubilized in their entirety and analyzed by LSC. Solubilization of samples was effected with a sodium hydroxide/methanol/Triton X-100 solution by overnight incubation and addition of

hydrogen peroxide to decolorize. Whole blood and faeces post-extraction solids were analyzed by combustion followed by LSC.

For metabolite profiling, pooled urine and cage wash samples were prepared for the 6-, 12-, 24- and 48-h time points by combining a percentage of the total weight of each individual animal sample for each collection time. Similarly, pooled faeces samples were prepared by combining 24- and 48-h time point samples. Pooled faeces samples were extracted three times with acetonitrile:water (3:1, v:v). Aliquots of pooled urine and cage wash samples, and faeces extracts, were analyzed by HPLC with in-line radioactive flow detection (RAD). The radiochemical purity of [^{14}C]DCSA in the dose preparation was determined by HPLC/RAD. Significant metabolites and parent DCSA were isolated from urine by HPLC and were identified by negative-ion LC-MS and LC-MS/MS analysis.

Findings:

General:

Analysis of the dose preparation before and after dosing indicated that it was stable during the time of dose administration. The average doses administered were 96.12 and 95.10 mg/kg bw for Groups 1 and 2, respectively.

Absorption:

DCSA was extensively absorbed from the gastrointestinal tract as evidenced by the large percentage of the dose excreted in the urine and small amount excreted in the faeces. Based on the amount of the dose excreted in the urine (94.96% of administered dose including cage washes which were urinary in nature) and carcass (0.25% of administered dose), the minimum average absorption of DCSA was greater than 95%. Absorption appeared to be rapid based on the fast elimination of the dose in the urine.

Distribution:

Radioactive residues in tissues seven days after dosing were very low, accounting for 0.01% of the administered dose or less for each individual tissue analyzed. Residues in the carcass averaged 0.25% of the administered dose (range of 0.14-0.47%). Detectable residues were observed in liver, kidney, and GI tract; no residues were detected in spleen, fat, and blood. A summary of the distribution of radioactivity in the tissues and organs at sacrifice is given in Table 5.8.1-2. Kidney contained the highest concentration of radioactivity with levels ranging from 0.080-0.103 □g-equivalents of DCSA per gram of tissue, followed by liver (0.030-0.090 □g-equiv/g tissue) and GI tract (0.024-0.036 □g-equiv/g tissue).

Table 5.8-2: Tissue residue concentrations for excretion/mass balance experiment

Matrix	Tissue Concentration (□g-equiv/g tissue)					
	Individual Animal				Mean	Std. Dev.
	195675	195807	195677	195678		
Liver	0.056	0.090	0.030	0.059	0.058	0.025
Kidney	0.080	0.093	0.085	0.103	0.090	0.010
GI Tract	0.024	0.024	0.036	0.025	0.027	0.006
Spleen	ND[1]	ND	ND	ND	ND	ND
Fat	ND	ND	ND	ND	ND	ND
Blood	ND	ND	ND	ND	ND	ND
Carcass	0.237	0.180	0.489	0.149	0.264	0.154

[1]ND = Not detected (below limit of detection).

Excretion:

Recovery of the administered dose for the Expired Air Metabolism experiment (Group 1) was 94.00% (range of 91.82-96.18%) (data not shown). Elimination in expired air as carbon dioxide or other volatiles was not a significant route of excretion. Only 0.03% of the administered dose was found in the expired air of Group 1 animals by 48 h after dosing.

Recovery of the administered dose for the Excretion/Mass Balance Experiment (Group 2) averaged 99.48% (range of 97.28-105.49%) (Table 5.8-3). DCSA was rapidly and efficiently eliminated primarily in the urine. Table 5.1-4 illustrates cumulative radioactivity recovered in excreta. An average of 94.96% (range of 92.73-100.14%) of the administered dose was recovered in the urine/cage wash by the end of day 7 (168 h) (cage wash radioactivity was urinary in nature). Faecal elimination was a minor route of excretion, with an average of 4.26% (range of 2.99-5.17%) of the administered dose excreted in faeces by the end of day 7 (168 h). Excretion of radioactivity was rapid with >96% of the administered dose excreted within 24 h and >98% of the administered dose excreted by 48 h.

Table 5.8-3: Total recovery of administered dose after [^{14}C]DCSA administration for excretion/mass balance experiment

	Percent of Administered Dose					
Matrix	**Individual Animal**				**Mean**	**Std. Dev.**
	195675	**195807**	**195677**	**195678**		
Urine	79.26	89.54	77.09	74.02	79.98	6.73
Cage	13.47	10.60	15.75	20.12	14.98	4.02
Faeces	4.60	5.17	4.29	2.99	4.26	0.92
Tissues[1]	0.23	0.18	0.48	0.15	0.26	0.15
Total	**97.56**	**105.49**	**97.61**	**97.28**	**99.48**	**4.01**

[1]Includes carcass.

Table 5.8-4: Cumulative recovery of administered dose after [^{14}C]DCSA administration for excretion/mass balance experiment

Urine + Cage Wash

	Percent of Administered Dose					
Time (h)	**Individual Animal**				**Mean**	**Std. Dev.**
	195675	**195807**	**195677**	**195678**		
6	55.93	67.26	64.11	47.75	58.76	8.76
12	75.07	90.04	83.89	64.50	78.38	11.10
24	90.06	99.51	91.05	90.69	92.83	4.48
48	92.35	100.05	91.90	93.66	94.49	3.78
72	92.52	100.09	92.57	94.00	94.79	3.60
96	92.58	100.11	92.68	94.08	94.86	3.56
120	92.61	100.13	92.72	94.10	94.89	3.56
144	92.63	100.14	92.78	94.11	94.92	3.55
168	92.73	100.14	92.83	94.14	94.96	3.51

Faeces

	Percent of Administered Dose					
Time (h)	**Individual Animal**				**Mean**	**Std. Dev.**
	195675	**195807**	**195677**	**195678**		
6	0.02	0.01	0.05	<0.005	0.02	0.02
12	0.02	0.02	0.05	0.01	0.03	0.02
24	3.66	4.57	3.85	1.00	3.27	1.56
48	4.52	5.10	4.20	2.93	4.19	0.92
72	4.57	5.15	4.26	2.97	4.24	0.92
96	4.58	5.16	4.27	2.99	4.25	0.92
120	4.59	5.16	4.28	2.99	4.26	0.92
144	4.59	5.17	4.29	2.99	4.26	0.92
168	4.60	5.17	4.29	2.99	4.26	0.92

Metabolism:

Metabolites were quantified in the pooled urine and faeces extracts of Group 2. Metabolites were also quantified in pooled cage washes since the washes contained a significant percentage of the dose. HPLC profiles of cage washes were very similar to urine HPLC profiles, indicating that cage wash radioactivity was derived from urine.

DCSA was poorly metabolized in the rat and was excreted largely unchanged primarily in urine. A summary of the distribution of radioactive residues in urine, cage wash, and faeces is given in Table 5.8-5. Unchanged DCSA accounted for approximately 82% of the administered dose. Two glucuronide conjugates of DCSA were identified, differing only in the position of glucuronidation (carboxyl moiety or phenol moiety). The position of glucuronidation of the metabolites was determined by their distinctive MS/MS fragmentation patterns – fragmentation by loss of CO_2 from the phenolic glucuronide indicated a free aromatic carboxyl group in that metabolite. DCSA phenolic and carboxyl glucuronides accounted for approximately 10% and 5%, respectively, of the administered dose. Several other very minor metabolites were observed, none of which constituted more than 1% of the dose. In total, more than 96% of the dose was identified. The proposed pathway for metabolism of DCSA in rats is shown in Figure 5.8-2.

Table 5.8-5: Distribution of radioactive residues in excreta for excretion/mass balance experiment

	Percent of Administered Dose		
Metabolite	**Urine + Cage Wash[1]**	**Faeces[2]**	**Total[3]**
3.57 min (unidentified)	ND[4]	0.03	0.03
6.71 min (unidentified)	0.02	ND	0.02
10.72 min (unidentified)	0.02	ND	0.02
13.73 min (unidentified)	0.15	ND	0.15
15.01 min (unidentified)	0.61	ND	0.61
DCSA phenolic	**10.36**	**ND**	**10.36**
DCSA carboxyl	**4.43**	**0.24**	**4.67**
19.27 min (unidentified)	ND	0.17	0.17
DCSA	**78.91**	**2.61**	**81.52**
Unextractable	NA[5]	1.11	1.11
Total Identified	**93.70**	**2.85**	**96.55**
Total	**94.49**	**4.16**	**98.65**

[1]Sum of values for 6-, 12-, 24-, and 48-h pooled urine and 6-, 12-, 24-, and 48-h pooled cage wash samples.
[2]Sum of values for 24- and 48-h pooled faeces samples.
[3]Sum of values for pooled urine+cage wash and pooled faeces.
[4]ND = Not Detected (below limit of detection). Non-detect values were calculated as 0 for summations.
[5]NA = Not Applicable.
DCSA phenolic glucuronide = β-D-Glucopyranuronic acid, 1-(3,6-dichloro-2-hydroxybenzoate)
DCSA carboxyl glucuronide = 2-Carboxy-3,6-dichlorophenyl-β-D-glucopyranosiduronic acid

Conclusion:

Following a single oral dose of 100 mg/kg bw, [^{14}C]DCSA was extensively absorbed by the rat and rapidly excreted with very little retention in tissues. Urinary excretion was the major route of elimination accounting for approximately 95% of the administered dose.

Levels of radioactivity in the tissues after 7 days were very low with kidney containing the highest levels of the dose.

Limited metabolism of DCSA occurred in the rat *via* glucuronidation at either the carboxylic acid or phenol moiety. Unchanged DCSA represented approximately 82% of the dose.

Figure 5.8-2: Proposed pathway for the metabolism of DCSA in rats

[EMBED ChemDraw.Document.5.0]

IIIA 10.2

DCSA carboxyl glucuronide

DCS

DCSA phenolic glucuronide

Revised by U.S. Environmental Protection Agency

Study Type: Metabolism and Pharmacokinetics

Report: IIA 5.8/2. Shah, J.F., McClanahan, R.H. (2009a). Pharmacokinetic Study of [^{14}C]DCSA in Sprague-Dawley Rats. Ricerca Biosciences, LLC, unpublished report No. MSL-20425/019911-1, study No. 06-98-M-5/XX-09-306. MRID 47899503.

Dates of Work: October 20, 2006 - November 1, 2006

Guidelines: EPA OPPTS 870.7485 Tier 2
Deviations: None
OECD 417 (partial – plasma kinetics, excretion and metabolism after oral gavage following repeated dosing in the diet at 5 dose levels)
Deviations: None
PMRA DACO 4.5.9

GLP: Yes

Executive Summary:

In a metabolism and pharmacokinetics study (MRID 47899503), unlabelled DCSA was administered in the diet for 14 days followed by a single oral gavage dose on the 15th day of [^{14}C]DCSA in corn oil to male and female rats (Sprague-Dawley Crl:CD® (SD)) at target dose levels of 42, 125, 250, 375, or 500 mg/kg bw.

There was a plateau in plasma C_{max} values following the [^{14}C]DCSA dose at dose levels above 125 mg/kg bw. Clearance of the dose is also limited, especially in females, even at the 125 mg/kg bw dose level, as evidenced by higher than dose-proportional increases in $AUC_{0-\infty}$ values with increasing dose. This leads to an increase in 24-h plasma concentration values at dose levels above 125 mg/kg bw. It appears that the breakpoint for the change in pharmacokinetic behaviour of DCSA occurs between the 125 and 250 mg/kg bw dose levels.

Following repeated dosing of males and females at 125 or 500 mg/kg bw, a single oral gavage dose of DCSA was well-absorbed and rapidly excreted, primarily in urine. While significant changes in pharmacokinetic parameters were observed with increasing dose level, only minor differences were observed in the metabolism and elimination of DCSA between dose levels or genders. Elimination at the high dose was somewhat slower than the low dose, likely due to delayed/saturated absorption at the high dose. Limited metabolism of DCSA occurred *via* glucuronidation at either the carboxylic acid or phenolic moieties. DCSA phenolic glucuronide and DCSA carboxyl glucuronide constituted 10-15% and 1.5-16% of the administered dose in the excreta, respectively.

No other metabolite exceeded 1% of the administered dose. The excretion pattern and metabolite profiles obtained in this study following repeated dosing at 125 or 500 mg/kg bw of both males and females are very similar to those obtained in the DCSA rat ADME study described in point 5.8.1 in which males only were dosed by single oral gavage with [^{14}C]DCSA at a 100 mg/kg bw dose level.

This study is classified totally reliable (acceptable/guideline) and satisfies the guideline requirement for a metabolism/pharmacokinetic study and should be evaluated with MRID 47899502.

Test conditions:

[Phenyl-U-^{14}C]DCSA (3,6-dichloro-2-hydroxybenzoic acid, refer to Figure 5.8.2-1 for structure) (Lot No. 6116-01B, radiochemical purity 96.9%, chemical purity 98.5%, specific activity 35.2 mCi/mmol, 6.29 MBq/mg, 377,507 dpm/µg); unlabelled DCSA (Lot No. GLP-0603-16958-T, purity 97.9%).

Figure 5.8.2-1: Structure of [^{14}C]DCSA test material

* Denotes uniform ^{14}C ring labelling

[EMBED ChemDraw.Document.5.0]

Unlabelled DCSA was administered in the diet (Lab Diet® Certified Rodent Diet, No. 5002 – Meal) for 14 days followed by a single oral gavage dose on the 15th day of [^{14}C]DCSA in corn oil (5 mL/kg bw) to male and female rats (Sprague-Dawley Crl:CD® (SD)) at one of five target dose levels: 42, 125, 250, 375, or 500 mg/kg bw (Groups 1-5, respectively) as outlined in Table 5.8-6. Animals of Groups 4 and 5 were acclimated to DCSA in the diet in a stepwise fashion due to potential palatability issues identified in a preliminary study to the 90-day rat study (study 5.8/6) below). For the ^{14}C dose, mixtures of radiolabelled and unlabelled DCSA were prepared at the appropriate specific activity to provide the target dose and approximately 250 µCi/kg bw of radioactivity. Actual dose levels achieved in the dietary exposure phase and by oral gavage are shown in Table 5.8-6.

Table 5.8-6: Study design

Group Number*	Number of Animals (M/F)	Target Dose Level (mg/kg)	Dose Days for Dosing in Feed**	Dose Level in Feed (ppm)	Dose Level Achieved during Feeding Phase (mg/kg bw, M/F)	^{14}C Dose Level Achieved (mg/kg bw, M/F)
1	8/8	42	0-13	500	39.14 / 40.36	43.08 / 42.77
2	8/8	125	0-13	1500	122.84 / 123.34	124.38 / 125.16
3	8/8	250	0-13	3000	246.72 / 248.76	242.24 / 253.29
4	8/8	375	0-6 7-13	3000 4500	365.45 / 370.67	364.36 / 372.36
5	12/12	500	0-4 5-8 9-13	3000 4500 6000	447.49 / 437.26	482.59 / 488.68

* The first day of dosing in feed is considered Study Day 0. All animals received the ^{14}C dose on Study Day 14.
** Each group was sub-divided into A and B sub-groups each containing half of the animals in the group (e.g., Group 2B contained 4 males and 4 females, Group 5A contained 6 males and 6 females).

A total of 44 male and 44 female rats were utilized for the study. The animals were 51-53 days old and weighed 198-246 g at the beginning of the dietary phase, and were 65-67 days old and weighed 256-381 g at the time of ^{14}C dosing. Animals in each group were subdivided into two sub-groups (A and B) to limit the number of bleedings of each animal. Blood samples (0.5 mL) were obtained from animals of Groups 1-5 at 2 h after completion of dietary exposure (2 h prior to the ^{14}C dose), and then at 0.5, 1, 2, 4, 6, 8, 12, 24 and 48 h after the ^{14}C dose, alternating between the A and B sub-groups. Thus, blood samples were obtained from sub-group A animals of Groups 1-5 at 2 h prior to the ^{14}C dose and at 1, 4, 8 and 24 h after ^{14}C dosing. Blood samples were collected from sub-group B animals of Groups 1-5 at 0.5, 2, 6, 12 and 48 h after ^{14}C dosing. Blood samples were obtained from the retro-orbital plexus except those obtained from the abdominal aorta at in-life termination (24 and 48 h after the ^{14}C dose for sub-groups A and B, respectively). Blood samples were processed to obtain plasma. Plasma from the first time point (2 h prior to ^{14}C dose) was analyzed by a quantitative method for DCSA to determine steady state DCSA concentrations following dietary exposure. Plasma from all other time points was analyzed for total radioactivity for determination of plasma kinetics. For animals in Group 2B (125 mg/kg bw) and Group 5B (500 mg/kg bw), urine, faeces, and cage washes were collected at 6, 12, 24, and 48 h after the ^{14}C dose, and these samples were analyzed for radiolabel content. Samples that contained sufficient amounts of radioactivity were separately pooled by time point, matrix, group, and gender. HPLC metabolite profiles were generated for pooled urine and cage washes and for extracts of the pooled faeces.

Analytical methods:
DCSA in rodent diet was determined by extraction with ethyl acetate/acetonitrile/trifluoroacetic acid (25/65/10 v/v/v) and analysis by HPLC with UV detection at 315 nm. The method was validated at nominal levels of 500, 3000 and 6000 ppm DCSA. Blood plasma samples collected following the dietary exposure phase were diluted with acetonitrile, vortexed and centrifuged. Aliquots of the supernatants were diluted with water and DCSA was quantified by negative-ion LC-MS/MS using a calibration curve generated with known concentrations of DCSA fortified into control rat plasma. Plasma samples collected following the ^{14}C dose were analyzed for total

radioactivity by liquid scintillation counting (LSC). Pharmacokinetic analysis of plasma total radioactivity data was conducted with WinNonlin Version 4.1 software.

Radioactivity in excreta was quantified by LSC. Urine and cage wash samples were analyzed directly by LSC. Faeces were homogenized with water and aliquots were analyzed by LSC after solubilization followed by decolorization with hydrogen peroxide. For metabolite profiling, pooled urine and cage wash samples were prepared for the 6-, 12-, 24- and 48-h time points by combining a percentage of the total weight of each individual animal sample for each collection time. Similarly, pooled faeces samples were prepared by combining 24- and 48-h time point samples. Pooled faeces samples were extracted three times with acetonitrile:water (3:1, v:v). Aliquots of pooled urine and cage wash samples, and faeces extracts, were analyzed by HPLC with in-line radioactive flow detection (RAD). The radiochemical purities of [^{14}C]DCSA in the dose preparations were determined by HPLC/RAD. Parent DCSA was identified by retention time comparison to the reference standard. Metabolites were identified by comparison of their HPLC retention times to those in the DCSA absorption, distribution, metabolism and elimination (ADME) study (study 5.8/1) in which the metabolites were conclusively identified by mass spectrometry.

Findings:

General:

One male animal of Group 5A (500 mg/kg bw) died after the 8-h blood collection possibly due to an overdose of CO_2/O_2 during the blood collection process. DCSA diet preparations were determined to be homogeneous, and DCSA was stable in the diet for at least 10 days. [^{14}C]DCSA dose preparations were homogeneous and stable during the ^{14}C dosing. Radioactive doses were within 5% of the target dose level for all groups. Achieved dietary dose levels (in mg/kg bw) were within 5% of the target dose level for all groups except Group 5 for which the achieved dose was 11-13% lower than the targeted 500 mg/kg bw.

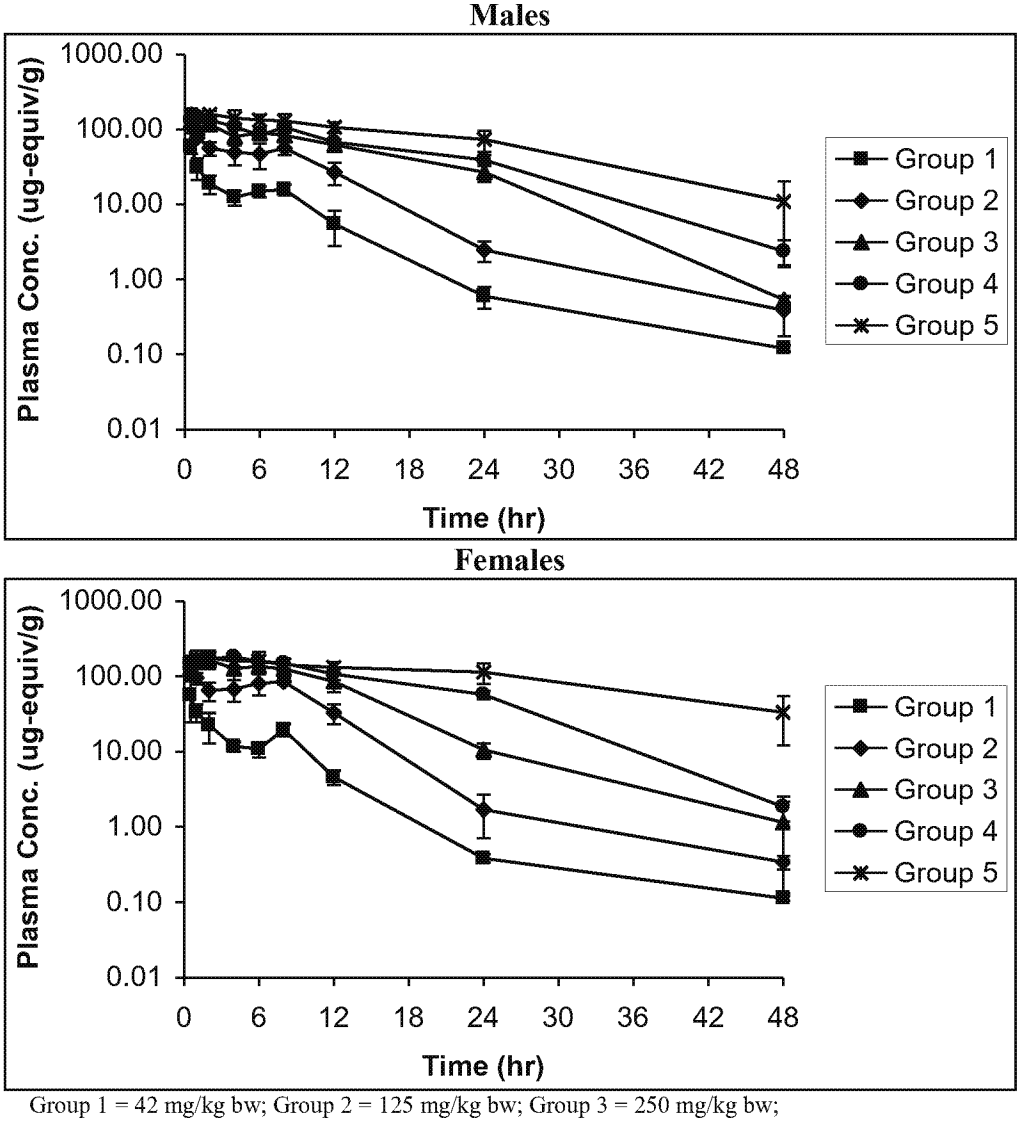
Plasma radioactivity levels:

Blood plasma total radioactivity concentration data are tabulated in Table 5.8-7 and are presented graphically in Figure 5.8-3.

Table 5.8-7: Mean plasma total radioactivity concentrations from a single oral dose of [^{14}C]DCSA following a 14-day dietary administration of DCSA

	Mean Plasma Concentration (µg-equiv/g)									
	Group 1		Group 2		Group 3		Group 4		Group 5	
	42 mg/kg bw		125 mg/kg bw		250 mg/kg bw		375 mg/kg bw		500 mg/kg bw	
Time (h)	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
0.5	58.789	57.006	107.632	105.828	157.460	161.134	135.958	150.662	151.230	148.257
1	31.073	33.651	77.559	95.162	103.421	172.245	147.080	160.392	145.585	175.571
2	18.922	22.761	56.235	65.348	116.484	166.035	133.497	171.949	159.145	176.574
4	12.426	11.757	49.319	67.726	79.564	126.988	108.849	182.020	141.829	158.670
6	14.839	10.931	46.917	81.067	90.230	136.560	83.202	158.811	132.748	160.490
8	15.758	19.621	56.020	85.303	82.729	126.066	105.843	149.488	128.981	142.438
12	5.458	4.606	26.890	32.890	61.599	85.537	67.388	107.519	106.717	132.284
24	0.596	0.386	2.450	1.708	26.908	10.567	39.028	57.341	72.912	113.778
48	0.120	0.113	0.389	0.341	0.534	1.151	2.376	1.860	10.990	33.391

Figure 5.8-3 Mean plasma total radioactivity concentrations after a single oral dose of ^{14}C[DCSA] following a 14-day dietary administration of DCSA



Group 1 = 42 mg/kg bw; Group 2 = 125 mg/kg bw; Group 3 = 250 mg/kg bw;
Group 4 = 375 mg/kg bw; Group 5 = 500 mg/kg bw

Pharmacokinetic parameters calculated from the total radioactivity concentration data are summarized in Table 5.8-8. After oral administration of [^{14}C]DCSA, the time (T_{max}) corresponding to peak plasma concentrations of total radioactivity (C_{max}) occurred at 0.5-2 h post-dose for males and 0.5-4 h post-dose for females, generally increasing with increasing dose. Total radioactivity concentrations then declined, but there was evidence of a secondary plasma maximum at 6-8 h, especially at the lower dose levels, possibly indicating some degree of enterohepatic cycling. Terminal plasma half-life values were relatively constant for both males and females for the 42, 125, and 250 mg/kg bw dose levels (5.67-5.81 h for males, 5.34-5.86 h for females) but increased for the 375 and 500 mg/kg bw dose levels. At the 500 mg/kg bw level, terminal plasma half-life values were 12.28 h for males and 20.18 h for females.

Table 5.8-8: Plasma kinetics (total radioactivity) of a single oral dose of [^{14}C]DCSA following a 14-day dietary administration of DCSA

Group Number	Dose Level (mg/kg bw)	Sex	T_{max} (h)	Half-life(h)	C_{max} (µg/g)	$AUC_{0-\infty}$ (h*µg/g)
1	42	Male	0.5	5.77	58.789	239.714
2	125 (3x)*	Male	0.5	5.81	107.632	824.018 (3.4x)*
3	250 (2x)	Male	0.5	5.67	157.460	1906.698 (2.3x)
4	375 (1.5x)	Male	1	7.41	147.080	2375.691 (1.2x)
5	500 (1.3x)	Male	2	12.28	159.145	3852.296 (1.6x)
Overall increase	11.9x					16.1x
1	42	Female	0.5	5.56	57.006	238.171
2	125 (3x)	Female	0.5	5.34	105.828	1076.376 (4.5x)
3	250 (2x)	Female	1	5.86	172.245	2262.133 (2.1x)
4	375 (1.5x)	Female	4	6.76	182.020	3516.410 (1.6x)
5	500 (1.3x)	Female	2	20.18	176.574	6015.526 (1.7x)
Overall increase	11.9x					25.3x

* values in parentheses are increase over previous (next lower) dose level

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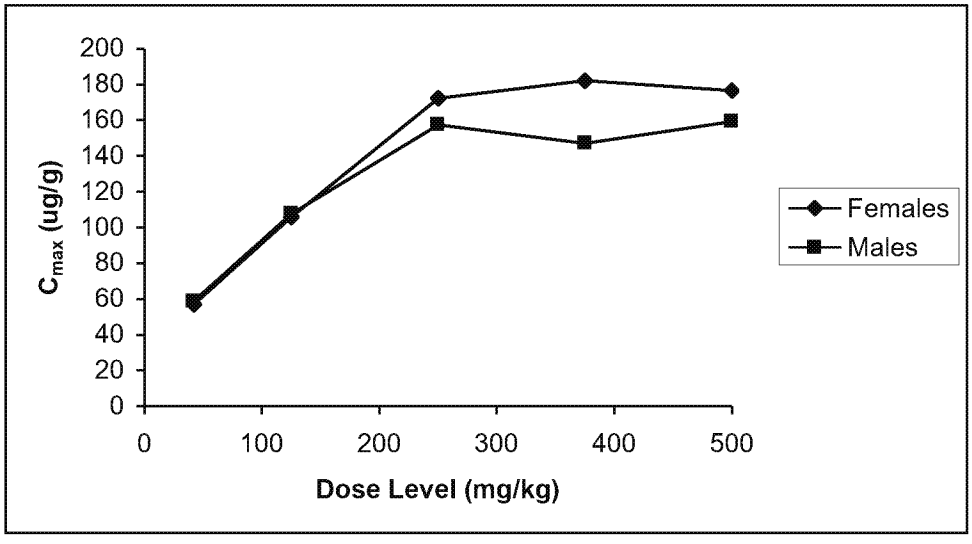
The extent of systemic exposure of rats to [^{14}C]DCSA-derived radioactivity, characterized by C_{max} and the area under the plasma-time curve ($AUC_{0-\infty}$), increased with increasing [^{14}C]DCSA dose over the dose range of 42-500 mg/kg bw. The relationship between C_{max} and dose level appeared to follow a linear but less than dose-proportional relationship for the 42 and 125 mg/kg bw dose levels, and C_{max} values then plateaued at approximately 150 µg-equiv/g for males and 180 µg-equiv/g for females for the 250, 375, and 500 mg/kg bw dose levels (refer to Figure 5.8-4). Thus, absorption appeared to be saturated at the 250 mg/kg bw dose level and higher.

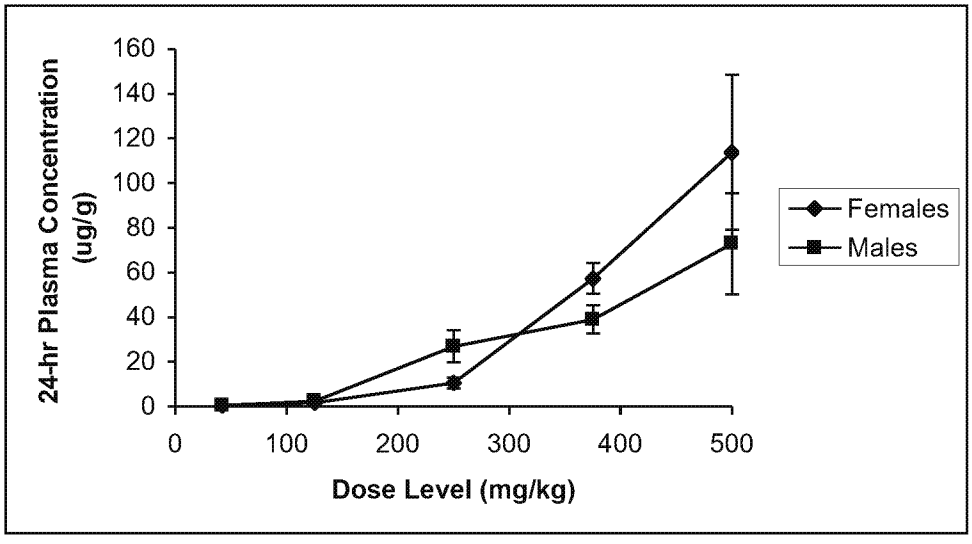
Figure 5.8-4. Mean plasma maximum concentration (C_{max}) after a single oral dose of [^{14}C]DCSA versus dose level



The increase in $AUC_{0-\infty}$ with increasing dose was higher than dose-proportional, especially for females, even for the 125 mg/kg bw dose level. For a 3.0-fold increase in dose from 42 to 125 mg/kg, $AUC_{0-\infty}$ values increased 3.4-fold for males and 4.5-fold for females. For an11.9-fold increase in dose level from 42 to 500 mg/kg bw, $AUC_{0-\infty}$ values increased 16.1-fold for males and 25.3-fold for females. The peak and extent of systemic exposure of rats to [^{14}C]DCSA-derived radioactivity were generally higher in females than in males, especially at the 250, 375, and 500 mg/kg bw dose levels, as indicated by higher C_{max} and $AUC_{0-\infty}$ values for females compared to males at the higher dose levels.

The relationship between mean 24-h plasma total radioactivity concentration values and dose level was approximately dose-proportional for the 42 and 125 mg/kg bw dose levels, and then the mean 24-h plasma total radioactivity concentration values increased quite dramatically in a greater than dose-proportional fashion for the 250, 375, and 500 mg/kg bw dose levels (refer to Table 5.8-7 and Figure 5.8-5). For a 3.0-fold increase in dose level from 42 to 125 mg/kg bw, mean 24-h plasma concentration values increased 4.1-fold for males and 4.4-fold for females. For an 11.9- fold increase in dose level from 42 to 500 mg/kg bw, mean 24-h concentration values increased 122-fold for males and 295-fold for females. Thus, following the [^{14}C]DCSA dose, plasma levels for Group 1 had decreased to approximately 1% of their maximum values (C_{max}) at 24 h after dosing, whereas plasma levels for Group 5 at 24 h after dosing had only decreased to approximately 46-64% of their maximum values. Based on the pharmacokinetic data, especially the 24-h plasma concentration data, a breakpoint in pharmacokinetic behaviour for DCSA in rats occurs between the 125 and 250 mg/kg bw dose levels.

Figure 5.8-5. Mean plasma total radioactivity 24 hours after a single oral dose of [^{14}C]DCSA versus dose level



DCSA plasma concentrations following dietary administration:

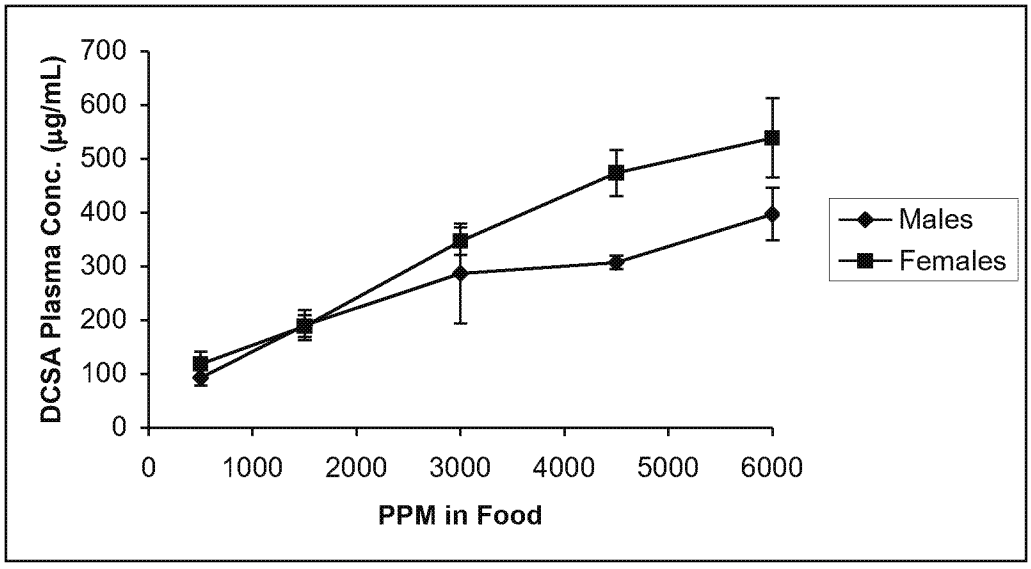
Plasma samples were collected 2 h after the termination of dietary exposure (2 h before dosing with [^{14}C]DCSA) and were analyzed for DCSA using an LC-MS/MS method. The relationship between the mean DCSA plasma concentration and dose level followed a similar, but less obvious, trend as the total plasma radioactivity C_{max} values (see Table 5.8-9 and Figure 5.8-6). The relationship between the plasma DCSA concentrations and dose level appeared to follow an approximately linear but less than dose-proportional relationship for the 500, 1500 and 3000 ppm (42, 125 and 250 mg/kg bw) dose levels. Plasma DCSA concentrations relative to dose for the 4500 and 6000 ppm (375 and 500 mg/kg bw) dose levels dropped considerably but did not fully plateau. At the 3000 ppm (250 mg/kg bw) dose level and above, plasma DCSA concentrations were somewhat higher for females than for males.

Table 5.8-9. Mean plasma concentration of DCSA following a 14-day dietary administration of DCSA

Group Number	Dose Level in Feed (ppm)	Sex	Mean DCSA Plasma Concentration (µg/mL)[1]
1A	500	Male	92.973
2A	1500	Male	190.770
3A	3000	Male	286.765
4A	4500	Male	307.082
5A	6000	Male	397.166
1A	500	Female	118.570
2A	1500	Female	188.674
3A	3000	Female	347.130
4A	4500	Female	473.618
5A	6000	Female	538.574

[1]Plasma samples were taken approximately 2 h after removal of treated feed and 2 h before administration of [^{14}C]DCSA.

Figure 5.8-6. Mean plasma concentration of DCSA following a 14-day dietary administration of DCSA versus dose level



Elimination:

The average recovery of the administered dose ranged from 71.08% to 84.90% (refer to Table 5.8-10) for males and females of Groups 2B and 5B. The recovery of the administered dose was not quantitative because excreta were collected for only 48 h and, more importantly, the animals were removed from the cages periodically for blood sampling for the pharmacokinetic phase of the study, resulting in some urination outside of the cages. Urinary excretion (urine+cage wash – cage wash radioactivity was urinary in nature) was the major route of elimination, accounting for 67.93-77.34% of the administered dose (88.89-95.57% of the recovered dose). Elimination *via* the faeces was a minor route of excretion, accounting for an average of 3.15-9.20% of the administered dose. A slightly higher percentage of the dose was excreted in the faeces for Group 5 (500 mg/kg bw dose) compared to Group 2 (125 mg/kg bw dose). Excretion of radioactivity was relatively rapid, with >70% of the dose, on average, excreted by 48 h after dosing (this value would likely be considerably higher if the animals had not been handled and quantitative recoveries had been achieved). Excretion of the dose was somewhat faster for the lower dose Group 2 animals compared to the higher dose Group 5 animals, likely due to saturated/delayed absorption at the higher dose. For Group 2, an average of 85.3-95.5% of the excreted dose was eliminated within 24 h, while for Group 5 an average of 52.7-60.0% of the excreted dose was eliminated within 24 h after the ^{14}C dose.

Absorption of DCSA was extensive at both dose levels as evidenced by the very small amount of the dose excreted in the faeces and the large amount of the dose excreted in the urine.

Table 5.8-10: Mean cumulative recovery of administered dose in excreta after a single oral dose of [^{14}C]DCSA following a 14-day dietary administration of DCSA

Group 2B (125 mg/kg bw)

	Percent of Administered Dose					
	Urine + Cage Wash		Faeces		Total	
Time (h)	Male	Female	Male	Female	Male	Female
6	17.04	16.79	0.31	<0.005	17.36	16.79
12	41.96	48.49	0.39	0.49	42.36	48.98
24	65.02	66.01	2.73	1.88	67.75	67.89
48	74.78	67.93	4.61	3.15	79.39	71.08

Group 5B (500 mg/kg bw)

	Percent of Administered Dose					
	Urine + Cage Wash		Faeces		Total	
Time (h)	Male	Female	Male	Female	Male	Female
6	12.06	11.92	0.15	0.09	12.21	12.01
12	26.40	23.35	0.30	0.24	26.70	23.58
24	47.53	43.15	2.19	1.55	49.71	44.71
48	73.59	77.34	9.20	7.56	82.79	84.90

Metabolism:

Metabolite profiles were generated for pooled urine, faeces and cage washes of males and females of Groups 2B and 5B (125 and 500 mg/kg bw, respectively). The similarity of the cage wash profiles to the urine profiles indicated that radioactivity in the cage washes was derived from urine. A summary of the distribution and identity of radioactive residues in excreta of Groups 2B and 5B is presented in Table 5.8-11.

Table 5.8-11: Distribution of radioactive residues in excreta

Group 2B (125 mg/kg bw)

	Percent of Administered Dose					
	Urine + Cage Wash		Faeces		Total	
Metabolite	Male	Female	Male	Female	Male	Female
3.57 min (unidentified)	0.29	0.31	ND	ND	0.29	0.31
3.82 min (unidentified)	ND[2]	ND	0.01	0.02	0.01	0.02
4.83 min (unidentified)	ND	ND	0.03	<0.005	0.03	<0.005
9.14 min (unidentified)	ND	ND	ND	ND	ND	ND
13.27 min (unidentified)	0.74	ND	ND	ND	0.74	ND
14.52 min (unidentified)	ND	ND	ND	ND	ND	ND
DCSA phenolic glucuronide	**10.29**	**10.52**	**ND**	**ND**	**10.29**	**10.52**
DCSA carboxyl glucuronide	**4.58**	**0.91**	**1.20**	**0.54**	**5.78**	**1.45**
19.52 min (unidentified)	ND	ND	0.10	0.06	0.10	0.06
DCSA	**58.88**	**56.16**	**2.25**	**1.35**	**61.13**	**57.51**
23.05 min (unidentified)	ND	0.02	ND	ND	ND	0.02
23.37 min (unidentified)	ND	ND	0.06	0.19	0.06	0.19
24.73 min (unidentified)	ND	ND	0.02	0.05	0.02	0.05
25.67 min (unidentified)	ND	ND	ND	ND	ND	ND
Unextractable	NA[1]	NA	0.55	0.45	0.55	0.45
Total Identified (% AD)[3]	**73.75**	**67.59**	**3.45**	**1.89**	**77.20**	**69.48**
Total Identified (% RD)[4]	**92.90**	**95.09**	**4.35**	**2.66**	**97.24**	**97.75**
Total	**74.78**	**67.92**	**4.22**	**2.66**	**79.00**	**70.58**

Group 5B (500 mg/kg bw)

	Percent of Administered Dose					
	Urine + Cage Wash		Faeces		Total	
Metabolite	Male	Female	Male	Female	Male	Female
3.57 min (unidentified)	0.13	0.20	ND	ND	0.13	0.20
3.82 min (unidentified)	ND[2]	ND	ND	<0.005	ND	<0.005
4.83 min (unidentified)	ND	ND	ND	<0.005	ND	<0.005
9.14 min (unidentified)	0.36	0.78	ND	ND	0.36	0.78
13.27 min (unidentified)	ND	ND	ND	ND	ND	ND
14.52 min (unidentified)	ND	ND	ND	ND	ND	ND
DCSA phenolic glucuronide	**13.17**	**15.11**	**0.03**	**ND**	**13.20**	**15.11**
DCSA carboxyl glucuronide	**11.04**	**8.67**	**5.09**	**4.15**	**16.13**	**12.82**
19.52 min (unidentified)	ND	ND	0.20	0.13	0.20	0.13
DCSA	**48.76**	**52.41**	**3.03**	**2.53**	**51.79**	**54.94**
23.05 min (unidentified)	ND	ND	ND	ND	ND	ND
23.37 min (unidentified)	ND	ND	0.05	0.03	0.05	0.03
24.73 min (unidentified)	ND	ND	ND	ND	ND	ND
25.67 min (unidentified)	0.12	0.17	ND	ND	0.12	0.17
Unextractable	NA[1]	NA	0.49	0.48	0.49	0.48
Total Identified (% AD)[3]	**72.97**	**76.19**	**8.15**	**6.68**	**81.12**	**82.87**
Total Identified (% RD)[4]	**88.14**	**89.74**	**9.84**	**7.87**	**97.98**	**97.61**
Total	**73.58**	**77.34**	**8.89**	**7.32**	**82.47**	**84.66**

[1]NA = Not Applicable.
[2]ND = Not Detected (below limit of detection). Non-detect values were calculated as 0 for summations.
[3]AD = Administered Dose. Values are total percent of the administered dose identified.
[4]RD = Recovered Dose. Values are total percent of the recovered dose identified.
DCSA phenolic glucuronide = β-D-Glucopyranuronic acid, 1-(3,6-dichloro-2-hydroxybenzoate)
DCSA carboxyl glucuronide = 2-Carboxy-3,6-dichlorophenyl-β-D-glucopyranosiduronic acid

Overall, unchanged DCSA was the major radioactive component in the excreta, accounting for 51.79-61.13% of the administered dose for Group 2B and 5B males and females. This corresponded to 77.00% and 80.91% of the recovered dose for Group 2B males and females, respectively, and 62.56% and 64.71% of the recovered dose for

Group 5B males and females, respectively. Limited metabolism of DCSA occurred *via* glucuronidation at either the phenol or carboxylic acid moieties. DCSA phenolic glucuronide accounted for 10.29-10.52% of the administered dose for Group 2B and 13.20-15.11% of the administered dose for Group 5B. DCSA carboxyl glucuronide accounted for 1.45-5.78% of the administered dose for Group 2B and 12.82-16.13% for Group 5B. Somewhat higher amounts of the carboxyl glucuronide were excreted at the high dose compared to the low dose. Larger amounts of the carboxyl glucuronide were observed in the faeces for Group 5B compared to Group 2B, possibly due to increased biliary excretion of the carboxyl glucuronide at the high dose. Besides the phenolic and carboxyl glucuronides, no other metabolite constituted more than 1% of the administered dose. Only minor differences between genders were observed for the metabolism of DCSA in rats.

Conclusion:

Pharmacokinetic data indicate that absorption of DCSA in rats is saturated at dose levels above 125 mg/kg bw as evidenced by a plateau in plasma C_{max} values following the [^{14}C]DCSA dose. More importantly, clearance of the dose is also limited, especially in females, even at the 125 mg/kg bw dose level, as evidenced by higher than dose-proportional increases in $AUC_{0-\infty}$ values with increasing dose. This leads to a dramatic increase in 24-h plasma concentration values at dose levels above 125 mg/kg bw. This study demonstrates that the breakpoint for the change in pharmacokinetic behaviour of DCSA occurs between the 125 and 250 mg/kg bw dose levels.

Following repeated dosing of males and females at 125 or 500 mg/kg bw, a single oral gavage dose of DCSA was well-absorbed and rapidly excreted, primarily in urine. While significant changes in pharmacokinetic parameters were observed with increasing dose level, only minor differences were observed in the metabolism and elimination of DCSA between dose levels or genders. Elimination at the high dose was somewhat slower than the low dose, likely due to delayed/saturated absorption at the high dose. Limited metabolism of DCSA occurred *via* glucuronidation at either the carboxylic acid or phenolic moieties. DCSA phenolic glucuronide and DCSA carboxyl glucuronide constituted 10-15% and 1.5-16% of the administered dose in the excreta, respectively. No other metabolite exceeded 1% of the administered dose. The excretion pattern and metabolite profiles obtained in this study following repeated dosing at 125 or 500 mg/kg bw of both males and females are very similar to those obtained in the DCSA rat ADME study described in point 5.8.1 in which males only were dosed by single oral gavage with [^{14}C]DCSA at a 100 mg/kg bw dose level.